

# An alternative approach to Michaelis-Menten kinetics that is based on the Renormalization Group: Comparison with the perturbation expansion beyond the sQSSA.

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## Abstract

We recall the perturbation expansion for Michaelis-Menten kinetics, beyond the standard quasi-steady-state approximation (sQSSA). Against this background, we are able to appropriately apply the alternative approach to the study of singularly perturbed differential equations that is based on the renormalization group (SPDERG), by clarifying similarities and differences. In the present demanding situation, we directly renormalize the bare initial condition value for the substrate. Our main results are: i) the 2nd order SPDERG uniform approximations to the correct solutions contain, up to 1st order, the same outer components as the known perturbation expansion ones; ii) the differential equation to be solved for the derivation of the 1st order outer substrate component is simpler within the SPDERG approach; iii) the approximations better reproduce the numerical solutions of the original problem in a region encompassing the matching one, because of the 2nd order terms in the inner components, calculated here for the first time to our knowledge; iv) the refined SPDERG uniform approximations, that we propose, give the correct asymptotically vanishing solutions, too, and allow to obtain results nearly indistinguishable from the solutions of the original problem in a large part of the whole relevant time window, even in the studied unfavourable kinetic constant case, for an expansion parameter value as large as  $\varepsilon = 0.5$ .

## 1 Introduction

Michaelis-Menten (MM) kinetics, that characterizes enzymatic reactions [1, 2, 3, 4], is a well known example in biomathematics [3, 4, 5, 6] of a system of ordinary differential equations (ODEs) characterized by two definitely different time scales. In fact, usually the complex reaches a *quasi-steady-state* of equilibrium with the substrate at the very beginning, whereas the generally experimentally observed part of the reaction happens on a time scale that can be as larger as several orders of magnitude [7]. Correspondingly, the standard quasi-steady-state approximation (sQSSA) [1, 2, 3, 4], in which the independent variables are chosen to be the substrate and the complex, with the time derivative of the complex taken to be zero, is the routinely considered starting point for investigating the system's dynamics. Nevertheless, different starting points are considered in the literature.

Actually, from the theoretical point of view [2, 3, 4, 8], MM kinetics is an example of boundary layer problem. Therefore, in the standard methods the solutions are approximated by the perturbation expansion (PE), in an appropriate parameter  $\varepsilon$ , of both the inner and the outer components of the two chosen independent variables, *i.e.*, of the solutions of systems of two ODEs with regular and singular perturbations, respectively, with the further imposition of the appropriate matching conditions (MCs), at each order in  $\varepsilon$ . Within this framework, both the sQSSA and the *total* quasi-steady-state approximation (tQSSA) [2, 9, 10, 11, 12, 13] represent the 0th order terms of the outer solutions.

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Noticeably, the PE beyond the tQSSA, in which the independent variables are chosen to be the total substrate (*i.e.*, the sum of the substrate and of the complex) and the complex, has the advantage that the value of the expansion parameter is lower than 1 (indeed, one has  $\varepsilon \leq 1/4$  in this case), for whatever kinetic constants and for whatever initial condition values (ICVs) [2, 9, 10, 11].

In the present work, we are interested in testing the correctness, in the case of MM kinetics, of the renormalization group approach to singularly perturbed differential equations (SPDERG) proposed by Chen, Goldenfeld and Oono in [14, 15]. Therefore, we focus on the simpler case of the PE beyond the sQSSA, by moreover making the usual choice for the expansion parameter (*i.e.*,  $\varepsilon = e_0/s_0$  with  $e_0$  the initial enzyme concentration and  $s_0$  the initial substrate one) [2, 3]. It can be shown that in this case the approximated solution in the substrate / complex phase space converges rapidly to the exact one, for reasonably small  $\varepsilon$  values [3], and it was indeed proved that this PE (for  $\varepsilon < 1$ ) converges uniformly for all times  $t \in [0, \infty)$  [4].

On the other hand, the problem is not obvious to be solved, because both of the practical difficulty in explicitly finding the outer components, and of the peculiarity in the MCs to be imposed, whose number of terms increases with the considered order in the expansion [4]. These difficulties are shared by the other standard PE methods for MM kinetics considered in the literature, such as in particular the one beyond the tQSSA [2, 9, 10, 11].

Even more because of these difficulties, after recalling the sQSSA and the main known results of the PE beyond it, we apply to MM kinetics the SPDERG alternative approach proposed in [14, 15], that is based on the renormalization group [16]. Noticeably, a preliminary attempt to apply this approach in the present case, in the even more demanding case of the tQSSA framework, has been presented in [17], but we follow here the different way of directly renormalizing the bare ICVs (for the sake of precision, the one of the substrate).

In fact, though this approach appears more generally applicable, it was in particular already shown successful for obtaining the leading terms of the uniform approximations (UAs) of the corresponding PE in other cases of dynamics characterized by the presence of a boundary layer [14, 15]. From the point of view of the present application, in which we calculate in detail the solutions up to 2nd order, we anticipate that the work could appear quite technical and cumbersome, nevertheless the calculations do not imply particular difficulties. On the other hand, this application to the demanding situation of MM kinetics, within the sQSSA framework, appears to allow to better understand both the working principles and the advantages / limits of the approach in similar cases, besides highlighting the analogies and the differences with the standard PE. Indeed, the study appears to make possible to get some insights on the working principles of the PE itself, too. Moreover, we obtain a more detailed knowledge of MM dynamics within the sQSSA framework, even only from the point of view of the 2nd order contributions to the inner solutions, that are presented here for the first time to our knowledge. From this last perspective, it is to be noticed that the calculation of the 2nd order inner contribution is not merely a technical exercise, but is fundamental for an in-depth understanding of the SPDERG scheme in the present case, as we will show in the paper.

The paper substantially consists of a first more introductive part, and of a second part in which we present and discuss our results. In detail, in the first part, we recall: in Section 2, the basis of MM kinetics and the sQSSA, by moreover introducing the particular values of the kinetic constants and the two ICV sets that we consider; in Section 3, the results of the standard PE beyond the sQSSA, up to the known 1st order; in Section 4, the basis of the alternative SPDERG approach proposed in [14, 15], with attention to the case of boundary layer problems. In the second part, we present and discuss: in Section 5, the derivation of the 1st order SPDERG UAs; in Section 6, the calculation of the 2nd order contributions to the SPDERG UAs; in Section 7, the refined 2nd order SPDERG UAs that can be proposed; in Section 8, the comparison between the different best UAs that we considered. Finally, in Section 9, we present our conclusions. The paper is moreover completed by three Appendices: in Appendix A and in Appendix B we report the 1st order inner solution for the complex, and the 2nd order ones both for the substrate and the complex for partially general ICVs, respectively; in Appendix C we verify that the ODE obtained at the 2nd order from the study of the substrate is indeed the same ODE that one finds from the study of the complex.

## 2 The sQSSA

The sQSSA represents a milestone in the mathematical modelling of enzymatic reactions [1, 2, 3, 4, 5, 6, 18, 19]. Here we just remind that the original paper by Michaelis and Menten dates back to more than one century ago [1], that the idea was already present in the previous paper by Henry [18], and that the approach was further developed in particular by Briggs and Haldane [19]. Schematically [2, 3], one is modelling the reaction between the enzyme  $E$ , the substrate  $S$ , the complex  $C$ , and the product  $P$ :



that is reversible in the first part and irreversible in the second one, with associated kinetic constants  $k_1$ ,  $k_{-1}$ , and  $k_2$ .

When introducing the concentrations  $e$ ,  $s$ ,  $c$  and  $p$ , respectively, by using the mass action law, we arrive to describe the process by means of a system of four 1st order ODEs. Then, within the standard framework [2, 3], we start by using the conservation law  $e + c = e_0 + c_0$ , that implies that the enzyme concentration  $e$  does only depend on the complex one  $c$ . Moreover, we observe that the product concentration  $p$  can be obtained from the complex concentration  $c$  by integrating (equivalently, one can use the other conservation law,  $s + c + p = s_0 + c_0 + p_0$ ). Finally, we assume that the concentrations of the complex and of the product are zero at the beginning for simplicity (*i.e.*,  $c_0 = p_0 = 0$ ).

Hence, we end up with the well known system of two 1st order ODEs that are to be obeyed by the variables  $s$  and  $c$  (with ICVs  $s(0) = s_0$  and  $c(0) = 0$ ):

$$\begin{cases} \dot{s}(t) &= k_1[s(t) + K_D] \left[ c(t) - \frac{e_0 s(t)}{s(t) + K_D} \right] \\ \dot{c}(t) &= -k_1[s(t) + K_M] \left[ c(t) - \frac{e_0 s(t)}{s(t) + K_M} \right], \end{cases} \quad (2)$$

where the dot means the time derivative. Here  $K_D = k_{-1}/k_1$  is the so-called dissociation constant, whereas  $K_M = (k_{-1} + k_2)/k_1$  is the parameter that is generally known as Michaelis constant. It can be further noticed that  $K_M - K_D = k_2/k_1 = K$  is the usual Van Slyke-Cullen constant [20]. Though the original kinetic constants,  $k_1$ ,  $k_{-1}$  and  $k_2$ , are the key physical parameters for the studied system,  $K_D$ ,  $K_M$  and  $K$  turn out to be the experimentally measurable ones, usually in particular on the basis of the sQSSA.

For the sake of clarity, a set of values for the kinetic constants can be chosen to be [2]:

$$k_1 = 1\mu M^{-1}s^{-1}; \quad k_{-1} = 4s^{-1}; \quad k_2 = 1s^{-1}; \quad (3)$$

As we will discuss in detail in the following, this choice already corresponds to a demanding case for applying the sQSSA, thus it is appropriate for the present analysis. In particular, these kinetic constants give  $K_D = 4\mu M$ ,  $K_M = 5\mu M$  and  $K = 1\mu M$ . We will moreover consider two sets of ICVs:

$$e_0^a = 1\mu M; \quad e_0^b = 5\mu M; \quad s_0^a = s_0^b = 10\mu M; \quad (4)$$

where we are taking different values of the initial enzyme concentration  $e_0$ , with  $e_0^b > e_0^a$ , by labelling  $a$  and  $b$  the two corresponding sets. We plot in [Fig. 1] the solutions of Eqs. (2), obtained by numerically integrating the system, for these two sets of values, respectively.

The logarithmic scale in the figures enhances the typical presence of two definitely different time scales, with the concentration of the complex  $c$  that evolves very rapidly at the beginning whereas it turns out to be in a *quasi-steady-state* or *pseudo quasi-equilibrium* in the second part, of definitely longer duration. Indeed, the presence of a *plateau*, in which the complex is really roughly constant, is well more evident in case  $a$ , and we will show in the following that this choice of the ICVs corresponds to a situation in which the sQSSA is expected to work better.

In fact, in the sQSSA one looks at the second part, by directly taking  $\dot{c} \sim 0$  in Eqs. (2) (which is obviously quite a coarse approximation, though it does not mean that the complex is assumed to be constant, but only that it depends algebraically on the substrate). Therefore, the approximation is the more correct the more the time scale corresponding to the rapid transient phase for the complex,  $\tau_c$ , is small with respect to the time scale  $\tau_s$ , that rules the slow decay of the substrate.

Though one can argue about more refined time scale evaluations [21], the most intuitive choice in order to roughly get the  $\tau_c$  order of magnitude corresponds to assume a constant substrate concentration

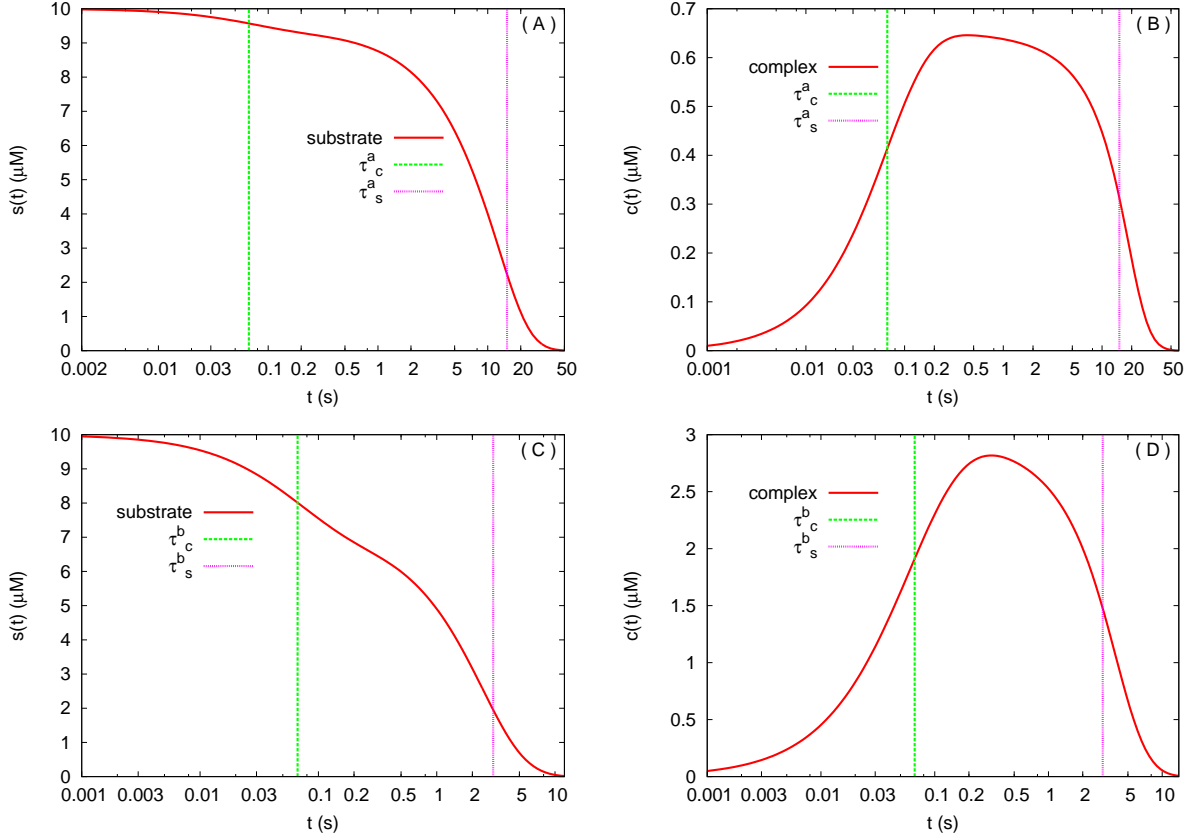


Figure 1: In A) and in C) we present the behaviour of the concentrations of the substrate  $s(t)$ , whereas in B) and in D) we present the ones of the complex  $c(t)$ , solutions of Eqs. (2), for the  $a$  and  $b$  sets of ICVs in (4), respectively. Notice that the time is in logarithmic scale. We plot the corresponding rough evaluations the two different time scales involved, too, with  $\tau_s$  describing the substrate decay time and  $\tau_c$  the complex saturation time (see the text for details).

in the first part, *i.e.*, a complex concentration that approximatively approaches exponentially its *plateau* value at the beginning [3],  $\dot{c} \sim -(c - c_{eq})/\tau_c$ , with  $\tau_c = 1/(k_1(s_0 + K_M))$ , that takes the same values  $\tau_c^a = \tau_c^b = 0.0667s$  in our case, for the  $a$  and  $b$  ICV sets respectively. On the other hand, when instead assuming  $\dot{c} \sim 0$  in the second part, one finds [3]  $\dot{s} \sim -k_2 e_0 s / (s + K_M) \sim -s/\tau_s$ , that approximatively describes the exponential decay to zero of the substrate in the end. Correspondingly, the substrate decay time is  $\tau_s = (s_0 + K_M)/(k_2 e_0)$ , that takes instead the two different values  $\tau_s^a = 15s$  and  $\tau_s^b = 3s$  in our case. Hence, the substrate decay time is well larger for the  $a$  set of ICVs than for the  $b$  one.

Let us now look more in detail at the behaviours displayed by the present numerical solutions of the original problem (2) when plotted in logarithmic scale. In fact, in the case of the substrate, one observes, both in [Fig. 1A] and in [Fig. 1B], the presence of three inflection points. Indeed, this feature appears to depend on the present choices of the kinetic constants and of the ICVs. We checked in particular the case  $k_1 = 1\mu M^{-1}s^{-1}$ ,  $k_{-1} = 10s^{-1}$  and  $k_2 = 20s^{-1}$  with the  $a$  set of ICVs and the present case with a definitely smaller value of  $e_0 = 0.1\mu M$ , and we found a (not shown) simpler behaviour, with only one inflection point in the curve for the substrate.

It is therefore even more interesting that, as can be seen from [Fig. 1], though the used approximations for evaluating the time scales can look questionable, in logarithmic scale the obtained estimation of  $\tau_s$  captures quite accurately the last (*i.e.*, the third) inflection point in the curve for the substrate, that therefore appears to be interpretable as the substrate decay time. On the other hand, the obtained estimation for  $\tau_c$  captures quite accurately the first inflection point in the curve for the complex, that therefore appears interpretable as the complex saturation time. In fact, the position of the third inflection point in the curve of the substrate roughly coincides with the one of the second inflection point in the curve for the complex. Correspondingly, both of the inflection points in the curve for the complex are captured by  $\tau_c$  and  $\tau_s$ , respectively (this is more evident in the case of the  $a$  set of ICV). Moreover, the complex saturation time  $\tau_c$  turns out to be quite near to the first inflection point in the curve for the substrate.

From this point of view, we notice that, in the previously recalled simpler considered cases, in

which the (not shown) curves for the substrates display single inflection points in logarithmic scale, their positions are more accurately captured by the corresponding substrate decay times  $\tau_s$ , and both  $\tau_c$  and  $\tau_s$  more accurately capture the positions of the two inflection points in the complex curves.

With the aim of deepening the analysis, we are led to the problem of adimensionalising the system in Eqs. (2). This involves the choice of the  $\varepsilon$  variable as the one giving the sQSSA condition  $\dot{c} \sim 0$  for  $\varepsilon = 0$ . In fact, this  $\varepsilon$  variable is clearly also the candidate for PEs that make possible to go beyond the sQSSA.

At this point, we remind that there are different approaches, in which for instance one chooses a different expansion parameter  $\varepsilon$  [2, 3, 21], or different independent variables at the beginning, such as in the already recalled tQSSA [2, 9, 10, 11, 12, 13], that turns out to make possible to describe a larger range of experimental situations and that could be therefore particularly useful in many cases.

In the present work, in order to test the SPDERG approach to MM kinetics, we focus on the sQSSA. In detail, within the two known possible adimensionalization schemes and the two corresponding different choices of the  $\varepsilon$  variable [2, 3, 21] in this case, we study the one that is more largely considered in the literature [2, 3, 5, 6, 4, 22], *i.e.*,  $\varepsilon = e_0/s_0$ .

In detail, one introduces the adimensional variables  $m = K_D/s_0$ ,  $M = K_M/s_0$  and one scales the time of a factor  $\delta$ ,  $t \rightarrow \delta t$  with  $\delta = k_1 e_0$ . The substrate concentration is made adimensional by taking  $\tilde{s}(t) = s(t)/s_0$ . Moreover, in the presently considered scheme, the complex concentration is made adimensional by taking  $\tilde{c}(t) = c(t)/e_0$  (which is a possible correct choice since, thanks to the first of the recalled conservation laws,  $c(t) \leq e_0$ ).

Correspondingly, one obtains the (singular with respect to  $\varepsilon$ ) system of ODEs:

$$\begin{cases} \dot{\tilde{s}}^{out}(t) &= [\tilde{s}^{out}(t) + m] \left[ \tilde{c}^{out}(t) - \frac{\tilde{s}^{out}(t)}{\tilde{s}^{out}(t) + m} \right] \\ \varepsilon \dot{\tilde{c}}^{out}(t) &= -[\tilde{s}^{out}(t) + M] \left[ \tilde{c}^{out}(t) - \frac{\tilde{s}^{out}(t)}{\tilde{s}^{out}(t) + M} \right]. \end{cases} \quad (5)$$

Here the label *out* refers to the fact that these are the ODEs that capture the long time behaviours, *i.e.*, the ones to be obeyed by the *outer* solution. With the present kinetic constant choice (3), we have  $m = 0.4$  and  $M = 0.5$ , respectively. On the other hand, the two sets of ICVs (4) yield two different values for  $\delta$  and for the expansion parameter  $\varepsilon$ :

$$\begin{aligned} \delta^a &= 1s^{-1}; & \delta^b &= 5s^{-1}; \\ \varepsilon^a &= 0.1; & \varepsilon^b &= 0.5; \end{aligned} \quad (6)$$

Thus, though the basic condition  $\varepsilon < 1$  is verified in both of the cases, we are in the situation  $\varepsilon^b > \varepsilon^a$ , that is expected to be the most appropriate for highlighting differences in the considered approximations.

The sQSSA corresponds clearly to take  $\varepsilon = 0$ . In fact [2, 3, 4], it can be rigorously interpreted as the 0th order term of an asymptotic expansion in  $\varepsilon$  of this example of singular perturbation, in which the original system is reduced to one ODE and one algebraic relation. For  $\varepsilon = 0$ , one has:

$$\begin{cases} \dot{\tilde{s}}_0^{out}(t) &= -\frac{M-m}{\tilde{s}_0^{out}(t) + M} \tilde{s}_0^{out}(t) \\ \tilde{c}_0^{out}(t) &= \frac{\tilde{s}_0^{out}(t)}{\tilde{s}_0^{out}(t) + M}. \end{cases} \quad (7)$$

It is to be noted that, in the numerator of the ODE to be obeyed by  $\tilde{s}_0^{out}(t)$ , the quantity  $M - m$  is just the adimensionalized Van Slyke-Cullen constant  $K/s_0$  [20].

The system is to be considered together with the ICV  $\tilde{s}_0^{out}(0) = 1$ , in fact implying that the other ICV is automatically fixed to  $\tilde{c}_0^{out}(0) = 1/(1 + M)$ .

The ODE for the adimensional substrate concentration can be solved explicitly [2, 23], by means of the Lambert function  $\omega(x)$  [24], that verifies the equation  $\omega(x)e^{\omega(x)} = x$ :

$$\tilde{s}_0^{out}(t) = M\omega(e^{-(M-m)t/M + 1/M}/M). \quad (8)$$

This solution does also satisfy the ICV  $\tilde{s}_0^{out}(0) = 1$ , since:

$$M\omega(e^{1/M}/M) = M\omega[\omega^{-1}(1/M)]. \quad (9)$$



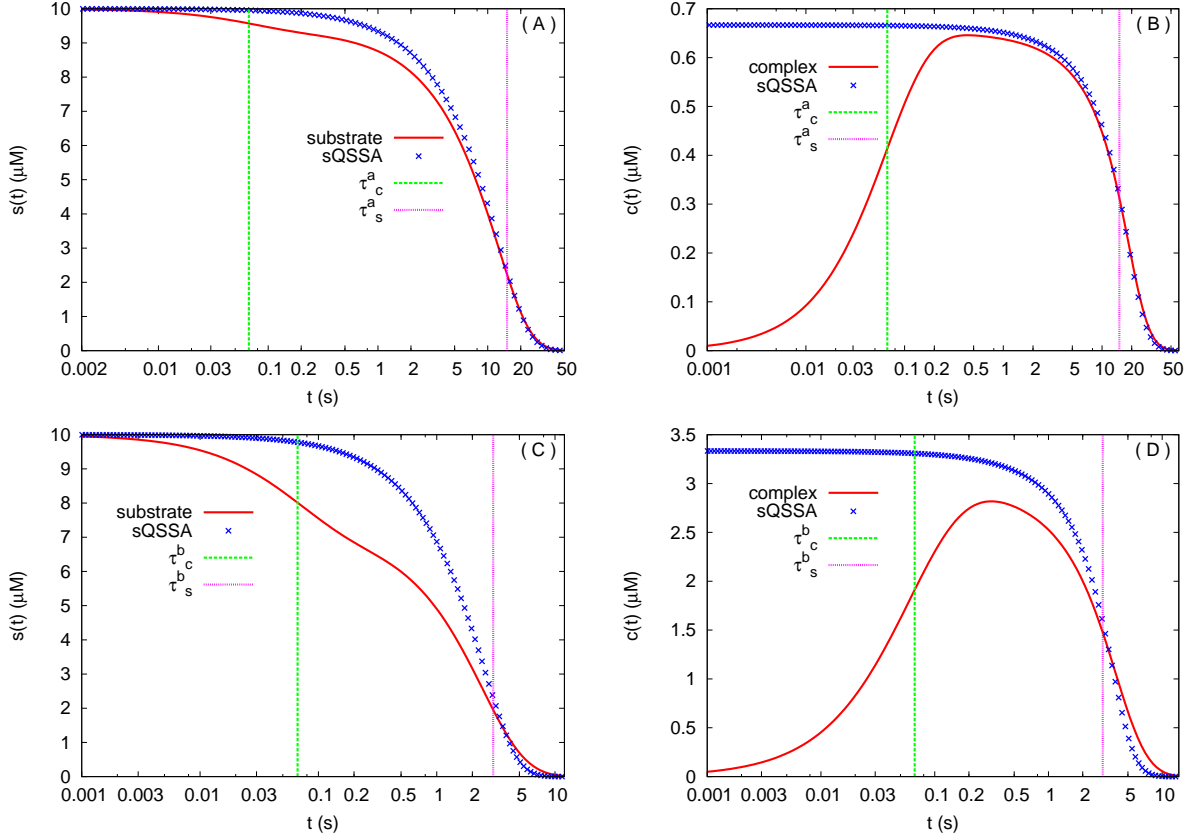


Figure 2: In A) and in C) we present the behaviour of the concentrations of the substrate  $s(t)$ , whereas in B) and in D) we present the ones of the complex  $c(t)$  for the  $a$  and  $b$  sets of ICVs given in (4), respectively. Hence, in A) and B) we are in the case with  $\varepsilon = \varepsilon^a = 0.1$ , whereas in C) and D) we are in the one with  $\varepsilon = \varepsilon^b = 0.5$ . We plot both the numerical solutions of Eqs. (2) already shown in the previous [Fig. 1], and the analytical solutions computed from the sQSSA ones (by using a standard numerical approximation for the Lambert function) as given in (8) and (10). We finally plot the corresponding rough evaluations of the two different time scales involved, too, with  $\tau_s$  describing the substrate decay time and  $\tau_c$  the complex saturation time. Notice that the time is in logarithmic scale.

Correspondingly, one gets:

$$\tilde{c}_0^{out}(t) = \frac{\omega(e^{-(M-m)t/M} + 1/M/M)}{\omega(e^{-(M-m)t/M} + 1/M/M) + 1}, \quad (10)$$

with, in particular,  $\tilde{c}_0^{out}(0) = 1/(1+M)$ , as expected.

Hence, by using a standard numerical approximation for the Lambert function, we obtain the behaviours of  $\tilde{s}_0^{out}(t)$  and  $\tilde{c}_0^{out}(t)$ , *i.e.*, the behaviours of the substrate and complex concentrations within the sQSSA. We show in [Fig. 2] our results for the two considered sets of ICVs, in comparison with the numerical solution of the original problem (2) (the same curves as in [Fig. 1]).

We note qualitatively that the failure of the approximation is well more evident for the  $b$  set of ICVs ([Fig. 2C] and [Fig. 2D]), as it could be expected, since  $\varepsilon^b > \varepsilon^a$ . In both cases, in these figures the logarithmic scale highlights that the complex very short time behaviour is not captured at all, a well known failure of the sQSSA (that is shared by the tQSSA, too). From this point of view, it is to be stressed that, as we already pointed out, whereas one can solve Eqs. (7) by choosing  $\tilde{s}_0^{out}(0) = 1$ , the original ICV for the complex concentration cannot *a priori* be satisfied within the present context. Therefore, the approximation is unable to predict the initial increase from zero of this quantity.

Noticeably, moreover, with the chosen kinetic constants and ICVs, the presence of more than one inflection point in logarithmic scale in the curve for the substrate (hence the short time qualitative behaviour of this quantity) is not reproduced at all. In fact, also the maximum reached by the complex during its evolution is lower than the ICV within the sQSSA in both of the considered cases. In particular, this last qualitative feature of a complex maximum lower than the sQSSA ICV is not at all observed in the previously recalled simpler considered cases, in which the (not shown) curves for

the substrates display a single inflection point in logarithmic scale, supporting the hypothesis that the various effects are related. Reasonably, it is for this reason that, in case *b*, it is naked-eye evident that the sQSSA fails in reproducing the long time behaviour, too (see [Fig. 2C] and [Fig. 2D]). Indeed, this case is even more demanding because of the quite large  $\varepsilon = \varepsilon^b = 0.5$ . In detail, one observes here sQSSA long time behaviours that tend to zero definitely more quickly than the numerical solutions of the original problem. Thus, as anticipated, this is a particularly demanding situation for correctly approximating MM dynamics and studying the standard PE that goes beyond the sQSSA.

### 3 The perturbation expansion beyond the sQSSA

The standard PE method, also in the case of MM kinetics [2, 3, 4, 5, 6], is based on mathematical results for systems of ODEs with both singular and regular perturbations. Precisely within this framework, system (5) is a well known example of singular perturbation, that is to be obeyed by the outer solutions of the original system. In the same framework, system (5) needs to be considered together with the system that one obtains by taking  $t \rightarrow \tau = t/\varepsilon$ , *i.e.*, in the opposite limit of short times, that is:

$$\begin{cases} \dot{\tilde{s}}^{in}(\tau) &= \varepsilon[\tilde{s}^{in}(\tau) + m] \left[ \tilde{c}^{in}(\tau) - \frac{\tilde{s}^{in}(\tau)}{\tilde{s}^{in}(\tau) + m} \right] \\ \dot{\tilde{c}}^{in}(\tau) &= -[\tilde{s}^{in}(\tau) + M] \left[ \tilde{c}^{in}(\tau) - \frac{\tilde{s}^{in}(\tau)}{\tilde{s}^{in}(\tau) + M} \right], \end{cases} \quad (11)$$

to be instead obeyed by the *inner* solutions.

Correspondingly [2, 3, 4, 5, 6], one searches solutions in the form:

$$\begin{cases} \tilde{s}^{in}(\tau) &= \sum_{i=0}^{\infty} \tilde{s}_i^{in}(\tau) \varepsilon^i; & \tilde{c}^{in}(\tau) &= \sum_{i=0}^{\infty} \tilde{c}_i^{in}(\tau) \varepsilon^i; \\ \tilde{s}^{out}(t) &= \sum_{i=0}^{\infty} \tilde{s}_i^{out}(t) \varepsilon^i; & \tilde{c}^{out}(t) &= \sum_{i=0}^{\infty} \tilde{c}_i^{out}(t) \varepsilon^i; \end{cases} \quad (12)$$

by requiring that  $\{\tilde{s}_i^{in}(\tau), \tilde{c}_i^{in}(\tau)\}$  satisfy the system (11) in the case of the inner solutions and that  $\{\tilde{s}_i^{out}(t), \tilde{c}_i^{out}(t)\}$  satisfy the system (5) in the case of the outer solutions, at each order in  $\varepsilon$ . One then imposes the appropriate MCs and takes, as UAs to the correct solutions at a given order in  $\varepsilon$ , the sum of the inner and of the outer solutions at that order minus the common terms [4].

The whole procedure appears therefore quite a standard PE method. For the sake of consistency, we also remind that one needs to impose appropriate MCs since, whereas one clearly takes inner solutions that also satisfy the ICVs (*i.e.*,  $\tilde{s}^{in}(0) = 1$  and  $\tilde{c}^{in}(0) = 0$ ), the ICVs of the outer solutions are to be determined from the behaviours of the inner ones in the large  $\tau$  limit, that have to correspond to their behaviours in the small  $t$  limit [2, 3, 4]. As we are going to discuss more in detail in the following, a particularly delicate point in the case of MM kinetics is represented just by the choice of the MCs, that actually, already at the 1st order, implies the need for considering MCs involving the 1st derivative of the outer solution, too [4].

In detail, in the present work, in the considered case of this kind of PE beyond the sQSSA with  $\varepsilon = e_0/s_0$ , we reproduce the calculations as also in [2, 3] at the 0th order, whereas we follow both the original paper by Heineken, Tsushiya and Aris [22] and the discussion in [4] for the complete 1st order contribution.

Let us first of all recall the 0th order inner solutions, obtained by setting  $\varepsilon = 0$  in Eqs. (11), that thereby solve:

$$\begin{cases} \dot{\tilde{s}}_0^{in}(\tau) &= 0 \\ \dot{\tilde{c}}_0^{in}(\tau) &= -[\tilde{s}_0^{in}(\tau) + M] \left[ \tilde{c}_0^{in}(\tau) - \frac{\tilde{s}_0^{in}(\tau)}{\tilde{s}_0^{in}(\tau) + M} \right], \end{cases} \quad (13)$$

with  $\tilde{s}_0^{in}(0) = 1$  and  $\tilde{c}_0^{in}(0) = 0$ . The solutions are [2, 3]:

$$\begin{cases} \tilde{s}_0^{in}(\tau) &= 1 \\ \tilde{c}_0^{in}(\tau) &= \frac{1}{1+M} \left[ 1 - e^{-(1+M)\tau} \right]. \end{cases} \quad (14)$$

At this point, we pass to consider the 0th order outer solution of the system (7) with ICV  $\tilde{s}_0^{out}(0) = \tilde{s}_0^{out*}$ , where  $\tilde{s}_0^{out*}$  needs to be determined by the MC for the substrate. Nevertheless, it is clear that, in the

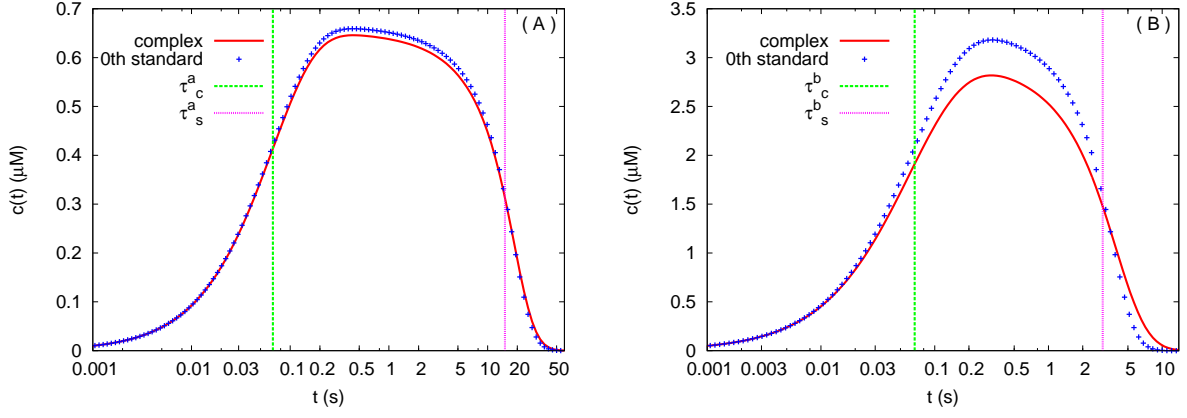


Figure 3: In A) we present the behaviour of the concentration of the complex  $c(t)$  for the  $a$  set of ICVs given in (4), whereas in B) we present the one for the  $b$  set. Hence, it is  $\varepsilon = \varepsilon^a = 0.1$  in the first case and  $\varepsilon = \varepsilon^b = 0.5$  in the second one. We plot both the numerical solutions of Eqs. (2) already shown in the previous figures, and the analytical solutions computed from the adimensional 0th order PE UAs (by using a standard numerical approximation for the Lambert function) as given in (16). We finally plot our corresponding rough evaluations of the two different time scales involved, too, with  $\tau_s$  describing the substrate decay time and  $\tau_c$  the complex saturation time. Notice that the time is in logarithmic scale.

relatively simple 0th order case, one expects that the substrate solution verifies  $\tilde{s}_0^{out*} = 1$ . In fact, with this choice, one has:

$$\begin{cases} \lim_{\tau \rightarrow \infty} \tilde{s}_0^{in}(\tau) &= \frac{1}{1+M} &= \lim_{t \rightarrow 0} \tilde{s}_0^{out}(t) \\ \lim_{\tau \rightarrow \infty} \tilde{c}_0^{in}(\tau) &= \frac{1}{1+M} &= \lim_{t \rightarrow 0} \tilde{c}_0^{out}(t). \end{cases} \quad (15)$$

Hence, consistently [4], the needed MC for the substrate satisfies the one for the complex, too.

In conclusion, the 0th order PE UAs (that we label  $u$ ), are given by [2, 3]:

$$\begin{cases} \tilde{s}_0^u(t) = \tilde{s}_0^{in}(t/\varepsilon) + \tilde{s}_0^{out}(t) - 1 \\ \tilde{c}_0^u(t) = \tilde{c}_0^{in}(t/\varepsilon) + \tilde{c}_0^{out}(t) - \frac{1}{1+M}. \end{cases} \quad (16)$$

We plot in [Fig. 3] our results on the complex concentrations (the UA for the substrate being coincident with the sQSSA for this quantity) for the two considered sets of ICVs, in comparison with the numerical solutions of the original problem (2) (the same curves as in [Fig. 1]), to show that this approximation already captures the most characteristic features of the whole system's dynamics, and in particular the rapid initial increase of the complex, too.

Nevertheless, the approximation over-evaluates the numerically obtained maximum values for the complex, as it is definitely more evident in case  $b$ , corresponding to the higher considered value of the expansion parameter,  $\varepsilon = \varepsilon^b = 0.5$  ([Fig. 3B]). On the other hand, as we already outlined, one could notice in the curves for the substrate ([Fig. 2A] and [Fig. 2C]) the sQSSA failure in capturing the presence of the first two inflection points (in logarithmic scale). In fact, it is now clear that this failure in correctly reproducing the qualitative short time substrate behaviour is shared by the 0th order PE UA, too. We underline once more that these observations appear explainable because the present choice of the kinetic constants (and of the initial values in case  $b$ ) makes the dynamical behaviour particularly complex.

Let us continue to sketch the procedure that is usually carried on, by recalling the calculation of the 1st order contribution [4, 22]. The 1st order inner solutions solve the system:

$$\begin{cases} \dot{\tilde{s}}_1^{in}(\tau) &= (1+m) \left[ \tilde{c}_0^{in}(\tau) - \frac{1}{1+m} \right], \\ \dot{\tilde{c}}_1^{in}(\tau) &= -(1+M) \tilde{c}_1^{in}(\tau) - \left[ \tilde{c}_0^{in}(\tau) - 1 \right] \tilde{s}_1^{in}(\tau). \end{cases} \quad (17)$$

Here, we already used  $\tilde{s}_0^{in}(\tau) = 1$ , thus the ICVs are  $\tilde{s}_1^{in}(0) = \tilde{c}_1^{in}(0) = 0$ . In fact, one has in particular to solve an ODE for  $\tilde{c}_1^{in}$  in the form  $\dot{y}(\tau) = -(1+M)y(\tau) + f(\tau)$ , whose solution is  $y(\tau) = a(\tau)e^{-(1+M)\tau}$ ,



with  $a(\tau) = \int_0^\tau f(z)e^{(1+M)z}dz$ . One obtains [4, 22]:

$$\begin{cases} \tilde{s}_1^{in}(\tau) &= -\frac{M-m}{1+M}\tau - \frac{1+m}{(1+M)^2} \left[ 1 - e^{-(1+M)\tau} \right] \\ \tilde{c}_1^{in}(\tau) &= -\frac{M(M-m)}{(1+M)^3}\tau - \frac{M(1+2m-M)}{(1+M)^4} \left[ 1 - e^{-(1+M)\tau} \right] + \\ &- \left[ \frac{(1-M)(1+m)}{(1+M)^3}\tau + \frac{M-m}{(1+M)^2} \frac{\tau^2}{2} \right] e^{-(1+M)\tau} + \\ &+ \frac{(1+m)}{(1+M)^4} e^{-(1+M)\tau} \left[ 1 - e^{-(1+M)\tau} \right]. \end{cases} \quad (18)$$

On the other hand, the 1st order outer solutions are more involved, since they solve the system (that consists once again of one ODE and of one algebraic relation):

$$\begin{cases} \dot{\tilde{s}}_1^{out}(t) &= \frac{M(M-m)}{[\tilde{s}_0^{out}(t) + M]^4} \tilde{s}_0^{out}(t) [\tilde{s}_0^{out}(t) + m] - \frac{M(M-m)}{[\tilde{s}_0^{out}(t) + M]^2} \tilde{s}_1^{out}(t) \\ \tilde{c}_1^{out}(t) &= \frac{M(M-m)}{[\tilde{s}_0^{out}(t) + M]^4} + \frac{M}{[\tilde{s}_0^{out}(t) + M]^2} \tilde{s}_1^{out}(t), \end{cases} \quad (19)$$

in which  $\tilde{s}_0^{out}(t)$  is reported, in terms of the Lambert function, in (8). One can verify that the solution for  $\tilde{s}_1^{out}(t)$  is given by [4]:

$$\tilde{s}_1^{out}(t) = \frac{\tilde{s}_0^{out}(t)}{\tilde{s}_0^{out}(t) + M} \left\{ \frac{m}{M} \log \left[ \frac{\tilde{s}_0^{out}(t) + M}{(1+M)\tilde{s}_0^{out}(t)} \right] - \frac{\tilde{s}_0^{out}(t) + m}{\tilde{s}_0^{out}(t) + M} \right\}. \quad (20)$$

Correspondingly, the 1st order outer solution for the complex is:

$$\tilde{c}_1^{out}(t) = \frac{\tilde{s}_0^{out}(t)}{[\tilde{s}_0^{out}(t) + M]^3} \left\{ m \log \left[ \frac{\tilde{s}_0^{out}(t) + M}{(1+M)\tilde{s}_0^{out}(t)} \right] + \frac{2M(M-m)}{\tilde{s}_0^{out}(t) + M} - M \right\}. \quad (21)$$

Indeed, if we were to neglect the *secular* terms (*i.e.*, the terms proportional to  $\tau$  in (18)), these solutions would also correctly satisfy the MCs, since one has:

$$\tilde{s}_1^{out}(0) = -\frac{1+m}{(1+M)^2} \quad \tilde{c}_1^{out}(0) = -\frac{M(1+2m-M)}{(1+M)^4}, \quad (22)$$

and these are also the constant terms in the 1st order inner solutions.

From the point of view of the present work, that aims to underline similarities and differences between the PE and the SPDERG approach, it appears important to stress that, within the standard method framework, one reasonably justifies the disappearance of the 1st order secular terms with the imposition of two term MCs [4]. In fact, these MCs involve the 1st order derivatives of the 0th order outer solutions, too. Moreover [4], one iteratively expects that the higher order divergences (hence in particular the presence of secular terms proportional to higher powers of  $\tau$  in the higher order inner solutions) could be absorbed by a possibly increasing number of terms in the corresponding MCs.

In detail, from Eqs. (7), one has:

$$\dot{\tilde{s}}_0^{out}(0) = -\frac{M-m}{(1+M)}; \quad \dot{\tilde{c}}_0^{out}(0) = -\frac{M(M-m)}{(1+M)^3}; \quad (23)$$

Correspondingly, it turns out to be verified a two term MCs [4], roughly summarizable as:

$$\begin{cases} \lim_{\tau \rightarrow \infty} \left\{ [\tilde{s}_0^{in}(\tau) + \varepsilon \tilde{s}_1^{in}(\tau)] - [\tilde{s}_0^{out}(0) + t \dot{\tilde{s}}_0^{out}(0) + \varepsilon \tilde{s}_1^{out}(0)] \right\} &= 0, \\ \lim_{\tau \rightarrow \infty} \left\{ [\tilde{c}_0^{in}(\tau) + \varepsilon \tilde{c}_1^{in}(\tau)] - [\tilde{c}_0^{out}(0) + t \dot{\tilde{c}}_0^{out}(0) + \varepsilon \tilde{c}_1^{out}(0)] \right\} &= 0. \end{cases} \quad (24)$$

Noticeably, it can be also proved [4] that the 1st order MCs, that we reported here, apply on a time interval (the matching region) that ranges from  $\varepsilon$  to  $\sqrt{\varepsilon}$ , and that the whole PE, with analogous MCs, approaches uniformly the correct solutions for  $t \in [0, \infty)$ .

Therefore, one takes as PE UAs to the solutions at the 1st order in  $\varepsilon$ :

$$\begin{cases} \tilde{s}_1^u(t) &= [\tilde{s}_0^{in}(t/\varepsilon) + \tilde{s}_0^{out}(t)] + \varepsilon [\tilde{s}_1^{in}(t/\varepsilon) + \tilde{s}_1^{out}(t)] - \left[ 1 - \varepsilon \frac{1+m}{(1+M)^2} - \frac{M-m}{(1+M)} t \right] \\ \tilde{c}_1^u(t) &= [\tilde{c}_0^{in}(t/\varepsilon) + \tilde{c}_0^{out}(t)] + \varepsilon [\tilde{c}_1^{in}(t/\varepsilon) + \tilde{c}_1^{out}(t)] + \\ &- \left[ \frac{1}{1+M} - \varepsilon \frac{M(1+2m-M)}{(1+M)^4} - \frac{M(M-m)}{(1+M)^3} t \right]. \end{cases} \quad (25)$$

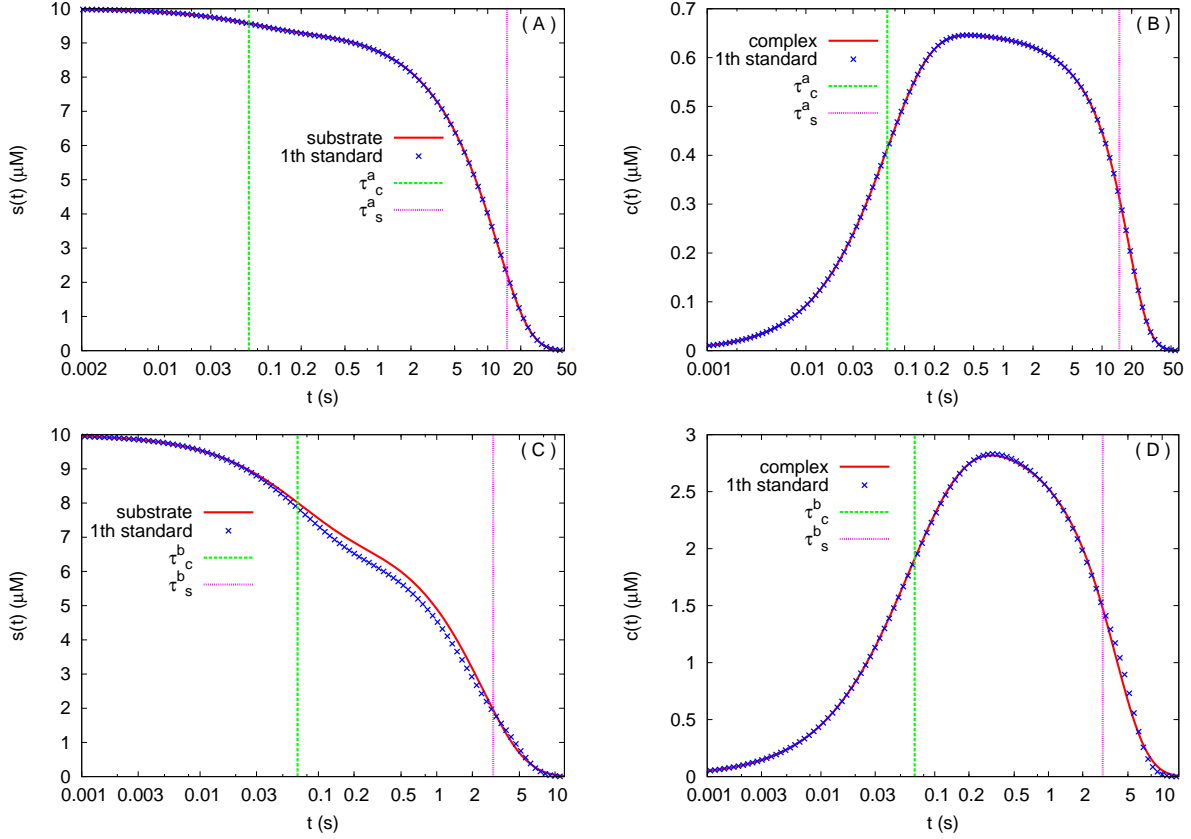


Figure 4: In A) and C) we present the behaviour of the concentrations of the substrate  $s(t)$ , whereas in B) and D) we present the ones of the complex  $c(t)$  for the  $a$  and  $b$  sets of ICVs given in (4), respectively. Hence, in A) and B) we are in the case with  $\varepsilon = \varepsilon^a = 0.1$ , whereas in C) and D) we are in the one with  $\varepsilon = \varepsilon^b = 0.5$ . We plot both the numerical solutions of Eqs. (2) already shown in the previous figures, and the analytical solutions computed from the 1st order PE UAs (by using a standard numerical approximation for the Lambert function) as given in (25). We finally plot our corresponding rough evaluations of the two different time scales involved, too, with  $\tau_s$  describing the substrate decay time and  $\tau_c$  the complex saturation time. Notice that the time is in logarithmic scale.

We plot in [Fig. 4] our results on the substrate and complex concentrations for the two considered sets of ICVs, in comparison with the numerical solutions of the original problem (2) (the same curves as in [Fig. 1]). The figures make evident that in the case  $a$ , ([Fig. 4A] and [Fig. 4B]) corresponding to a small  $\varepsilon = \varepsilon^a = 0.1$ , the 1st order PE UAs are indistinguishable, within the plot precision (in fact, they are nearly indistinguishable within our numerical precision, too), from the correct solution on the whole relevant time interval, despite of the particularly demanding situation, that is characterized by the presence of three inflection points in the curve for the substrate and of a sQSSA ICV for the complex that is higher than its correct maximum.

On the other hand, in case  $b$ , that corresponds to the higher  $\varepsilon = \varepsilon^b = 0.5$ , there is a naked-eye detectable difference for the substrate ([Fig. 4C]), for  $t \sim 0.1 \div 2s$ . This region actually encompasses the matching one, which is expected to extend here from  $t = \varepsilon^b/\delta^b = 0.1s$  to  $t = \sqrt{\varepsilon^b}/\delta^b \simeq 0.14s$ . Moreover, again in case  $b$ , when looking carefully at [Fig. 4D], one can notice that the complex tends to zero still slightly too rapidly. In fact, also the maximum reached by the complex during its evolution is still slightly over-evaluated. Both of these last observations, that concern the 1st order UA for the complex within the PE framework, will appear more evident in the following [Fig. 8C] and [Fig. 8D].

## 4 The SPDERG approach to boundary layer problems

The SPDERG approach and its connections with the renormalization group, with attention to the context of boundary layer problems, have been extensively reviewed in [17], and a preliminary attempt to apply it to MM kinetics within the tQSSA framework is presented in that work, too. Moreover, a recent review of the general method, in a context that is instead different from the boundary layer one, is presented in [25]. Here we limit ourselves to recall in some detail the original discussion in [14, 15],

where the approach was generally proposed for multiple-scale problems, in the case of the boundary layer ones. Indeed, in those works, the method was already shown successful in some of these last cases.

As MM kinetics [2, 3, 4], these problems [8] are generally characterized by a boundary layer of a given small thickness (that is here  $O(\varepsilon)$ , but that could also be, for instance,  $O(\sqrt{\varepsilon})$ ), in which the solution is rapidly varying. Correspondingly, in order to predict the system's dynamics, one needs to solve singularly perturbed ODEs and one usually resorts to a standard PE method.

To start to sketch the general SPDERG procedure in these cases, one starts from the singular 2nd order ODE for  $y(t)$  and makes the transformation  $t \rightarrow \tau = t/\varepsilon$ , thereby studying the ODE to be obeyed by the inner solution  $Y(\tau)$ . The approach basically consists in focusing on the large  $\tau$  behaviour of this solution, by outlining the secular terms, with the aim of proposing first of all the correct renormalization of the integration constants, that allows to eliminate them.

At this point, we anticipate that in the end one usually gets [14, 15] a physically meaningful solution, that also contains the leading terms of the outer expansion, and that is therefore to be interpreted as the UA within this approach, to be compared with the one of the standard PE methods. As we are going to verify in detail in the present application, this approximation is expected [14, 15] to work even better than the corresponding UA solution of the PE at the previous order in the matching region. Moreover, as we are going to discuss, it appears possible to make an analogy between the basic mechanism of the SPDERG approach to boundary layer problems and the one that allows to impose the correct MCs in the standard methods.

More precisely [15], one studies the solution  $Y(\tau)$  with given boundary conditions at a given time  $\tau_0$  and *renormalizes* the  $Y_{div}(\tau)$  part of this solutions that contains the Cauchy data, the other leading order terms and the terms that do not tend to zero or to a constant value at large times. This is obtained by renormalizing the corresponding *bare* integration constants (*i.e.*, the Cauchy data of the problem, though these integration constants can also more generally *contain* them in some given form), let us say  $A_0(\tau_0)$  and  $B_0(\tau_0)$ , as  $A_0(\tau_0) \rightarrow Z_1 A(\lambda)$ ,  $B_0(\tau_0) \rightarrow Z_2 B(\lambda)$ . In fact, the renormalization constants  $Z_1$  and  $Z_2$  depend both on  $\tau_0$  and  $\lambda$ , their basic role being to change, in the part to be renormalized of the function, the initial dependence of the Cauchy data on  $\tau_0$  in a dependence on the arbitrary time  $\lambda$ .

In detail, they are assumed to have the expansion (with  $a_0 = b_0 = 1$ ):

$$Z_1 = \sum_{n=0}^{\infty} a_n(\tau_0, \lambda) \varepsilon^n; \quad Z_2 = \sum_{n=0}^{\infty} b_n(\tau_0, \lambda) \varepsilon^n. \quad (26)$$

Since one can always take  $(\tau - \tau_0) = (\tau - \lambda) + (\lambda - \tau_0)$ , the secular terms in  $(\lambda - \tau_0)$  are correspondingly absorbable in appropriate redefinitions of  $A_0$  and  $B_0$ , that have to be made by correctly choosing the coefficients  $\{a_n\}, \{b_n\}$ . As it will become clear in the application that we are going to present, one can usually safely expect to be able to absorb in the coefficients of the renormalization constants also other possible secular terms, for instance those corresponding to higher powers of  $(\tau - \tau_0)$ . In particular, we will see in the present 2nd order calculations that, when writing  $(\tau - \tau_0)^2 = (\tau - \lambda)^2 + (\lambda - \tau_0)^2 + 2(\tau - \lambda)(\lambda - \tau_0)$ , only the second term is to be absorbed, whereas the last one is cancelled by the previously chosen 1st order renormalization coefficients, in the same way as in a case that is considered in [15] (see Section B in that work).

Summarizing [14, 15], the basic hypothesis, that is analogous to the scaling one in the renormalization group theory, is that the bare quantities need to be renormalized in such a way that the solution turns out to be independent of the arbitrary time  $\lambda$ , as it has reasonably to be. Hence,  $\lambda$  plays a key role in the whole approach. Intuitively, one can therefore think to  $\lambda$  as the equivalent of the *unknown time* at which the matching needs to be obeyed, and from this point of view there is an analogy with the imposition of the MCs in the standard PE methods. Nevertheless, though this analogy can be useful, as we will show, it is not to be taken too rigorously, particularly in the present peculiar case in which the 1st order matching requires two term conditions, and one expects that the correct matching could require even more terms at higher orders [4].

Going back to the recalling of the main details of the approach in the case of boundary layer problems [15], the appropriate choice of the coefficients of the renormalization constants, and the appropriate redefinitions of  $A_0$  and  $B_0$ , turns out in the replacement of these bare quantities with  $A(\lambda)$  and  $B(\lambda)$ , and in the corresponding change of variable  $\tau_0 \rightarrow \lambda$  in  $Y_{div}(\tau)$ . Then, on this  $\lambda$ -depending  $Y_{div}$ , one imposes the *scaling* condition  $dY_{div}/d\lambda = 0$ , that allows to obtain the 1st order ODEs to be obeyed by  $A(\lambda)$  and  $B(\lambda)$ . Finally, the change of variable  $\lambda \rightarrow \tau$  in the renormalized  $Y_{div}$ , in which the integration

constants are replaced by the solutions of these equations, and the imposition of the original boundary conditions of the problem, allow to get the physically meaningful result.

## 5 Results and discussion: i) First order contribution

Here we use, for the first time to our knowledge, the SPDERG approach to study MM kinetics beyond the sQSSA, whose known PE UAs we already recalled in Sections 2 and 3. In order to make the presentation as clear as possible, we stress from the beginning that we introduce a procedure that is slightly different from the one considered in [14, 15]. In fact, we make explicit the dependence from the ICVs in the solutions, thus outlining that, though in principle one can renormalize even general integration constants that contain them, the bare quantities that are basically to be renormalized are just the ICVs of the problem.

We notice first of all that one can rewrite the system (11) for the inner solutions in the form of a 2nd order ODE (see in particular [13] for the first study of MM kinetics in terms of a 2nd order ODE) to be obeyed by the adimensional substrate concentration:

$$\ddot{\tilde{s}}^{in}(\tau) - \frac{[\dot{\tilde{s}}^{in}(\tau)]^2}{\tilde{s}^{in}(\tau) + m} + [\tilde{s}^{in}(\tau) + M] \dot{\tilde{s}}^{in}(\tau) + \varepsilon \left[ \frac{m}{\tilde{s}^{in}(\tau) + m} \dot{\tilde{s}}^{in}(\tau) + (M - m) \tilde{s}^{in}(\tau) \right] = 0, \quad (27)$$

with ICVs (Cauchy data)  $\tilde{s}^{in}(0) = 1$  and  $\dot{\tilde{s}}^{in}(0) = -\varepsilon$ . Analogously, one can write the same system (11) in the form of a 2nd order ODE to be obeyed by the adimensional complex concentration:

$$[1 - \tilde{c}^{in}(\tau)] \ddot{\tilde{c}}^{in}(\tau) + [\dot{\tilde{c}}^{in}(\tau)]^2 + M \tilde{c}^{in}(\tau) \dot{\tilde{c}}^{in}(\tau) + \varepsilon [1 - \tilde{c}^{in}(\tau)]^2 [\dot{\tilde{c}}^{in}(\tau) + (M - m) \tilde{c}^{in}(\tau)] = 0, \quad (28)$$

with ICVs (Cauchy data)  $\tilde{c}^{in}(0) = 0$  and  $\dot{\tilde{c}}^{in}(0) = 1$ .

Actually, the physically meaningful solutions of these 2nd order ODEs are identical to the solutions of the original system (11). In fact, at the 1st order in  $\varepsilon$ , the equations are satisfied by  $\tilde{s}^{in}(\tau) = \tilde{s}_0^{in}(\tau) + \varepsilon \tilde{s}_1^{in}(\tau)$  and  $\tilde{c}^{in}(\tau) = \tilde{c}_0^{in}(\tau) + \varepsilon \tilde{c}_1^{in}(\tau)$ , with  $\tilde{s}_0^{in}(\tau)$ ,  $\tilde{c}_0^{in}(\tau)$  given by (14), and  $\tilde{s}_1^{in}(\tau)$ ,  $\tilde{c}_1^{in}(\tau)$  given by (18), respectively.

Therefore, instead of attempting to apply the approach to these 2nd order ODEs, we study the SPDERG (*i.e.*, *renormalization group*, that we label *rg*) adimensional substrate and complex concentrations,  $\tilde{s}^{rg}(\tau)$  and  $\tilde{c}^{rg}(\tau)$ , that are solutions of the system (11). The difference, with respect to the  $\tilde{s}^{in}(\tau)$  and  $\tilde{c}^{in}(\tau)$  that we previously considered within the PE method, is that here the ICVs are given at a time  $\tau_0$ , to be considered in principle different from zero, as  $\tilde{s}^{rg}(\tau_0) = \tilde{s}^*$  and  $\tilde{c}^{rg}(\tau_0) = \tilde{c}^*$ , respectively. Indeed, these  $\tilde{s}^*$  and  $\tilde{c}^*$  values are the bare quantities to be renormalized, whereas the original ICVs of the problem, *i.e.*,  $\tilde{s}(0) = 1$  and  $\tilde{c}(0) = 0$ , will be taken into account after the renormalization procedure.

For the sake of clarity, at the cost of being somehow repetitive, at the 1st order in  $\varepsilon$ , we search once again solutions in the form:

$$\begin{cases} \tilde{s}^{rg}(\tau) = \tilde{s}_0^{rg}(\tau) + \varepsilon \tilde{s}_1^{rg}(\tau) \\ \tilde{c}^{rg}(\tau) = \tilde{c}_0^{rg}(\tau) + \varepsilon \tilde{c}_1^{rg}(\tau). \end{cases} \quad (29)$$

In these formulas,  $\tilde{s}_0^{rg}(\tau)$  and  $\tilde{c}_0^{rg}(\tau)$  solve as usual the 0th order system (13), but the ICVs are given at  $\tau = \tau_0$ , and their values are  $\tilde{s}_0^{rg}(\tau_0) = \tilde{s}_0^*$  and  $\tilde{c}_0^{rg}(\tau_0) = \tilde{c}_0^*$ . These solutions are:

$$\begin{cases} \tilde{s}_0^{rg}(\tau) = \tilde{s}_0^* \\ \tilde{c}_0^{rg}(\tau) = \tilde{c}_0^* e^{-(\tilde{s}_0^* + M)(\tau - \tau_0)} + \frac{\tilde{s}_0^*}{\tilde{s}_0^* + M} \left[ 1 - e^{-(\tilde{s}_0^* + M)(\tau - \tau_0)} \right]. \end{cases} \quad (30)$$

On the other hand,  $\tilde{s}_1^{rg}(\tau)$  and  $\tilde{c}_1^{rg}(\tau)$  need to solve the 1st order system (that is slightly different from Eqs. (17) because of the presence of  $\tilde{s}_0^* \neq 1$ ):

$$\begin{cases} \dot{\tilde{s}}_1^{rg}(\tau) = (\tilde{s}_0^* + m) \tilde{c}_0^{rg}(\tau) - \tilde{s}_0^* \\ \dot{\tilde{c}}_1^{rg}(\tau) = -(\tilde{s}_0^* + M) \tilde{c}_1^{rg}(\tau) - [\tilde{c}_0^{rg}(\tau) - 1] \tilde{s}_1^{rg}(\tau), \end{cases} \quad (31)$$

with ICVs, at  $\tau = \tau_0$ ,  $\tilde{s}_1^{rg}(\tau_0) = \tilde{s}_1^*$  and  $\tilde{c}_1^{rg}(\tau_0) = \tilde{c}_1^*$ . We obtain, in the case of the substrate:

$$\tilde{s}_1^{rg}(\tau) = \tilde{s}_1^* - (M - m) \frac{\tilde{s}_0^*}{\tilde{s}_0^* + M} (\tau - \tau_0) +$$

$$- \frac{\tilde{s}_0^* + m}{(\tilde{s}_0^* + M)^2} [\tilde{s}_0^* - \tilde{c}_0^*(\tilde{s}_0^* + M)] \left[ 1 - e^{-(\tilde{s}_0^* + M)(\tau - \tau_0)} \right], \quad (32)$$

whereas the solution for the complex,  $\tilde{c}_1^{rg}(\tau)$ , is given in Appendix A.

Let us start by studying  $\tilde{s}^{rg}(\tau)$ . Following [15], we look at  $\tilde{s}_{div}^{rg}(\tau)$ , that contains just the ICVs (*i.e.*, the terms that give  $\tilde{s}^{rg}(\tau_0) = \tilde{s}_0^* + \varepsilon \tilde{s}_1^*$ ), the other possible leading terms at this order (absent in the present case), and the secular terms (here the one proportional to  $(\tau - \tau_0)$  in  $\tilde{s}_1^{rg}(\tau)$ ). Therefore, we write:

$$\tilde{s}^{rg}(\tau) = \tilde{s}_{div}^{rg}(\tau) + \varepsilon \mathcal{R}_1^s(\tau) + O(\varepsilon^2), \quad (33)$$

by grouping in  $\mathcal{R}_1^s$  all the sub-leading terms that tend to zero or to a constant value in the large time limit and that are not to be renormalized. One has:

$$\tilde{s}_{div}^{rg}(\tau) = \tilde{s}_0^* + \varepsilon \tilde{s}_1^* - \varepsilon(M - m) \frac{\tilde{s}_0^*}{\tilde{s}_0^* + M} (\tau - \tau_0). \quad (34)$$

As previously recalled, the SPDERG approach [14, 15], at this point, is based on writing  $(\tau - \tau_0) = (\tau - \lambda) + (\lambda - \tau_0)$ , by correspondingly assuming, in the present case, that the secular term proportional to  $(\lambda - \tau_0)$  can be absorbed in an appropriate redefinition of the bare constants. In detail, by labelling  $\tilde{s}_0^{rg*}$  and  $\tilde{s}_1^{rg*}$  the contributions to the renormalized ICV at the 0th and at the 1st order, respectively, we put  $\tilde{s}_0^* = Z_{s_0} \tilde{s}_0^{rg*}(\lambda)$  and  $\tilde{s}_1^* = Z_{s_1} \tilde{s}_1^{rg*}(\lambda)$ . The renormalization constants,  $Z_0^s$  and  $Z_1^s$ , are assumed to have the same  $\varepsilon$ -expansions as the ones given in (26). This expansion, at the 1st order in  $\varepsilon$ , that we considered here, implies  $\tilde{s}_0^* = [1 + \varepsilon z_{s_0,1}(\tau_0, \lambda)] \tilde{s}_0^{rg*}(\lambda)$  and  $\tilde{s}_1^* = \tilde{s}_1^{rg*}(\lambda)$ . Thus, the present secular term can be absorbed by choosing:

$$z_{s_0,1}(\tau_0, \lambda) = \frac{(M - m)}{\tilde{s}_0^{rg*}(\lambda) + M} (\lambda - \tau_0). \quad (35)$$

Therefore, we end up to study:

$$\tilde{s}_{div}^{rg}(\tau, \lambda) = \tilde{s}_0^{rg*}(\lambda) + \varepsilon \tilde{s}_1^{rg*}(\lambda) - \varepsilon(M - m) \frac{\tilde{s}_0^{rg*}(\lambda)}{\tilde{s}_0^{rg*}(\lambda) + M} (\tau - \lambda). \quad (36)$$

Hence, by imposing the scaling condition [14, 15],  $d\tilde{s}_{div}^{rg}(\tau, \lambda)/d\lambda = 0$ , we get the 1st order ODEs to be obeyed by the renormalized quantities.

In detail, at the 1st order in  $\varepsilon$ , we obtain:

$$\frac{d\tilde{s}_0^{rg*}(\lambda)}{d\lambda} + \varepsilon \frac{d\tilde{s}_1^{rg*}(\lambda)}{d\lambda} - \varepsilon(M - m) \frac{\tilde{s}_0^{rg*}(\lambda)}{\tilde{s}_0^{rg*}(\lambda) + M} = 0, \quad (37)$$

and, correspondingly:

$$\begin{cases} \frac{d\tilde{s}_0^{rg*}(\lambda)}{d\lambda} &= -\varepsilon(M - m) \frac{\tilde{s}_0^{rg*}(\lambda)}{\tilde{s}_0^{rg*}(\lambda) + M} \\ \frac{d\tilde{s}_1^{rg*}(\lambda)}{d\lambda} &= 0. \end{cases} \quad (38)$$

Consistently, when making the further transformation [15]  $\lambda \rightarrow \tau = t/\varepsilon$ , the first of these equations is just the ODE to be obeyed by the 0th order outer adimensional substrate concentration, that we encountered in Eqs. (7). Its solution is therefore  $\tilde{s}_0^{rg*}(t) = \tilde{s}_0^{out}(t)$ , that is given in (8) by means of the Lambert function, and that also satisfies the ICV  $\tilde{s}_0^{rg*}(0) = 1$ .

Clearly, this result already suggests that, also in the present particular demanding case of MM kinetics, the SPDERG approach proposed in [14, 15] turns out to be able to reproduce the leading order terms of the PE UAs.

On the other hand, the result  $d\tilde{s}_1^{rg*}(\lambda)/d\lambda = 0$  is here to be interpreted as the verification that the  $\tilde{s}_1^{rg*}$  term can be neglected at this order. In fact, we find  $\tilde{s}_1^{rg*} = \text{const} = 0$  by imposing the ICV (more correctly the ICV, that is fixed here to the value  $\tilde{s}(0) = 1$ , should be imposed on the solution at the end, but this is not influential in the present case, since one generally has  $\mathcal{R}(0) = 0$  for the appropriately calculated contribution of the parts not to be renormalized of the inner solutions).

Finally, we get the renormalized result:

$$\tilde{s}_1^{rg,u}(t) = \tilde{s}_0^{out}(t) + \varepsilon \mathcal{R}_1^s(t/\varepsilon) + O(\varepsilon). \quad (39)$$



Notice that, when we were considering  $\tilde{s}^{rg}(\tau)$  as the inner solution, in (33), before applying the SPDERG approach, we knew that it was correct up to order  $O(\varepsilon^2)$ . Here, instead, the renormalized  $\tilde{s}_1^{rg,u}(t)$  (that for this reason we label  $rg,u$ , by moreover making explicit that it is a 1st order approximation) is to be interpreted as the SPDERG UA to the solution of the original problem, clearly bearing in mind that, from this point of view, it is correct only up to order  $O(\varepsilon)$ . Actually, it is only expected to contain the leading order terms of the outer solution, as one can check that is indeed the case, by comparing it with (25).

Notice moreover that the terms in  $\mathcal{R}_1^s$ , that did not need to be renormalized, are to be evaluated in  $\tau_0 = 0$ , by using the correct ICVs for the bare constants (*i.e.*,  $\tilde{s}_0^* = 1$ ,  $\tilde{s}_1^* = 0$ ,  $\tilde{c}_0^* = 0$ ). Thus, the SPDERG 1st order UA to the solution for the adimensional substrate concentration does instead contain the 1st order terms of the PE UA to the correct solution that comes from  $\tilde{s}_1^{in}(t/\varepsilon)$ . On the other hand, correspondingly, the usual asymptotically vanishing solution behaviour that one finds when applying the recalled PE method to MM kinetics (*i.e.*,  $\lim_{t \rightarrow \infty} \tilde{s}(t) = 0$ ), is not verified here (one has  $\lim_{t \rightarrow \infty} \tilde{s}_1^{rg,u}(t) = O(\varepsilon)$ ). We will better discuss this failure of the present application of the SPDERG approach in the following, by proposing a way to bypass it, too.

Let us now study the complex. First of all, despite of the presence of a large number of terms in (62) (the formula given in Appendix A), that describes the behaviour of the 1st order inner adimensional complex concentration within the SPDERG approach,  $\tilde{c}_1^{rg}(\tau)$ , the part of the function to be renormalized up to the 1st order in  $\varepsilon$  is as manageable as in the case of the substrate.

In detail, we write the same kind of expression as in (33):

$$\tilde{c}^{rg}(\tau) = \tilde{c}_{div}^{rg}(\tau) + \varepsilon \mathcal{R}_1^c(\tau) + O(\varepsilon^2), \quad (40)$$

by collecting in  $\mathcal{R}_1^c$  all the sub-leading terms that tend to zero or to a constant value in the large time limit. Correspondingly, we find:

$$\tilde{c}_{div}^{rg}(\tau) = (\tilde{c}_0^* + \varepsilon \tilde{c}_1^*) e^{-(\tilde{s}_0^* + M)(\tau - \tau_0)} + \frac{\tilde{s}_0^*}{\tilde{s}_0^* + M} - \varepsilon \frac{M(M - m)}{(\tilde{s}_0^* + M)^3} \tilde{s}_0^*(\tau - \tau_0). \quad (41)$$

In fact, here we considered explicitly the terms that give the original ICV, (*i.e.*,  $\tilde{c}_{div}^{rg}(0) = \tilde{c}_0^* + \varepsilon \tilde{c}_1^*$ ). Nevertheless, both the 0th order contribution and the 1st order one to it are exponentially suppressed in the large time limit. Therefore, one can hypothesize (see an analogous case in [15], Section C) that also  $\tilde{c}_0^{rg*}(\lambda)$ , when renormalizing, should obey an ODE of the kind  $d\tilde{c}_0^{rg*}(\lambda)/d\lambda = 0$ . Hence, one expects to find  $\tilde{c}_0^{rg*}(\lambda) = \text{const} = 0$  when imposing the ICV (fixed here to  $\tilde{c}(0) = 0$ ) at the end of the renormalization procedure. Moreover, one also expects  $\tilde{c}_1^{rg*}(\lambda) = \text{const} = 0$ , a result that is even more predictable at this order, since we already verified that  $\tilde{s}_1^{rg*}$  gives no contribution in the case of the substrate. Correspondingly, we assume that these terms can be neglected in the present application of the SPDERG approach to MM kinetics.

Therefore, the function to be studied is further reduced to:

$$\tilde{c}_{div}^{rg}(\tau) \Big|_{\substack{\tilde{c}_0^*=0 \\ \tilde{c}_1^*=0}} = \frac{\tilde{s}_0^*}{\tilde{s}_0^* + M} - \varepsilon(M - m) \frac{M\tilde{s}_0^*}{(\tilde{s}_0^* + M)^3} (\tau - \tau_0). \quad (42)$$

As already recalled, in the context of the standard method, the MC that is verified by the adimensional substrate concentration does consistently also satisfy the matching of the inner and outer adimensional complex concentrations. For this reason, on the basis of the previously sketched analogy, we find not particularly surprising that, when making the transformations  $\{\tau_0 \rightarrow \lambda, \tilde{s}_0^* \rightarrow \tilde{s}_0^{rg*}(\lambda)\}$ , the scaling condition  $d\tilde{c}_{div}^{rg}(\tau, \lambda)/d\lambda = 0$  gives again the same ODE to be obeyed by  $\tilde{s}_0^{rg*}(\lambda)$  as the one that we just found in the study of  $\tilde{s}_{div}^{rg}(\tau, \lambda)$  (*i.e.*, Eqs. (38)). Hence (with the transformation  $\lambda \rightarrow \tau = t/\varepsilon$ ), we get again  $\tilde{s}_0^{rg*}(t) = \tilde{s}_0^{out}(t)$ . One can check in particular that, since  $d[\tilde{s}_0^*/(\tilde{s}_0^* + M)]/d\tilde{s}_0^* = M/(\tilde{s}_0^* + M)^2$ , when renormalizing the bare constant  $\tilde{s}_0^*$  by using the value of the coefficient  $z_{s_0,1}$  that is already fixed by (35), the contribution of the first term to the order  $\varepsilon$  is exactly equal to  $\varepsilon(\lambda - \tau_0)M(M - m)\tilde{s}_0^{rg*}/(\tilde{s}_0^{rg*} + M)^2$ , and therefore it suitably absorbs the secular term that is present in this case.

By recalling that  $\tilde{c}_0^{out}(t) = \tilde{s}_0^{out}(t)/(\tilde{s}_0^{out}(t) + M)$  from Eqs. (7), the obtained SPDERG 1st order UA to the correct solution is:

$$\tilde{c}_1^{rg,u}(t) = \tilde{c}_0^{out}(t) + \varepsilon \mathcal{R}_1^c(t/\varepsilon) + O(\varepsilon), \quad (43)$$

with, as for the substrate, the terms in  $\mathcal{R}_1^c$  to be evaluated in  $\tau_0 = 0$ , by using the correct ICVs for the bare constants (*i.e.*,  $\tilde{s}_0^* = 1$ ,  $\tilde{s}_1^* = \tilde{c}_0^* = \tilde{c}_1^* = 0$ ).

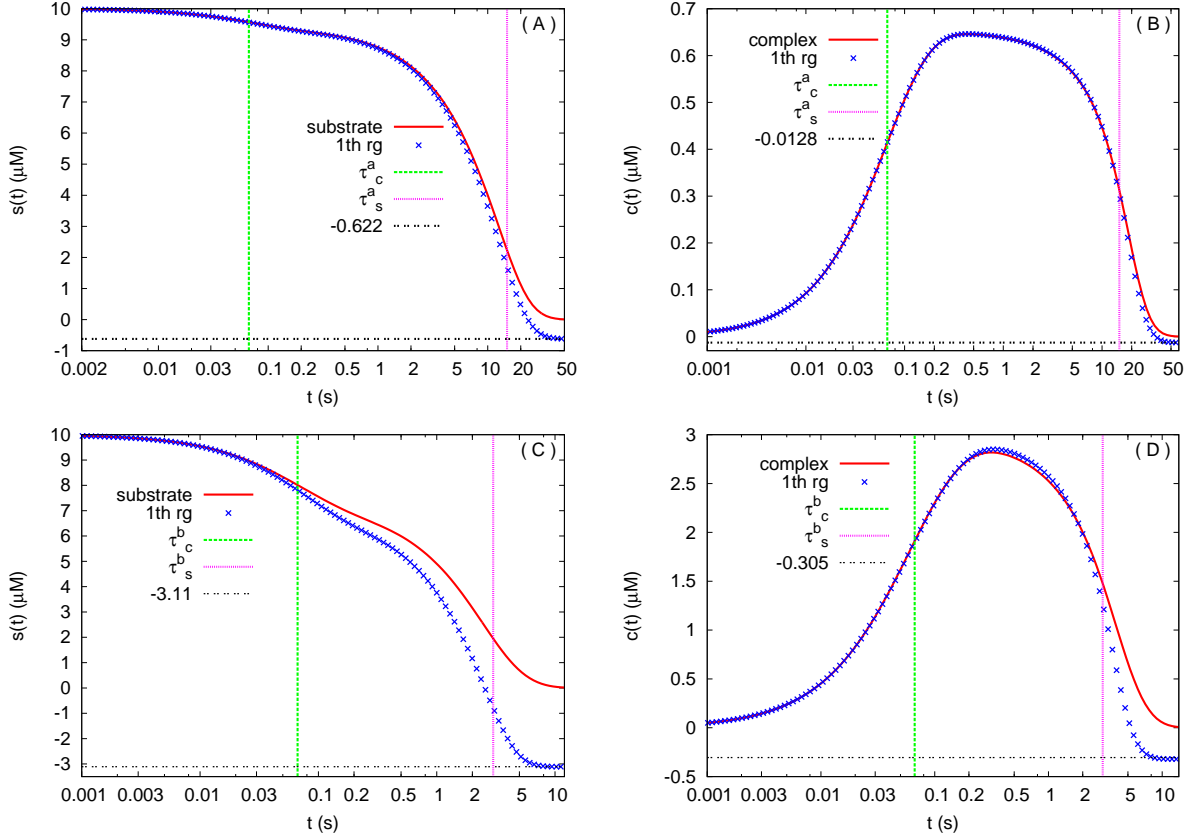


Figure 5: In A) and C) we present the behaviour of the concentrations of the substrate  $s(t)$ , whereas in B) and D) we present the ones of the complex  $c(t)$  for the  $a$  and  $b$  sets of ICVs given in (4), respectively. Hence, in A) and B) we are in the case with  $\varepsilon = \varepsilon^a = 0.1$ , whereas in C) and D) we are in the one with  $\varepsilon = \varepsilon^b = 0.5$ . We plot both the numerical solutions of Eqs. (2) already shown in the previous figures, and the analytical solutions computed from the SPDERG 1st order UAs (with a standard numerical approximation for the Lambert function), as given in (44). We plot moreover the (physically meaningless) limits for  $t \rightarrow \infty$  of the analytical solutions:  $s_0^a \tilde{s}_{1,\infty}^{rg,u} \simeq -0.622$ ,  $e_0^a \tilde{c}_{1,\infty}^{rg,u} \simeq -0.0122$ , and  $s_0^b \tilde{s}_{1,\infty}^{rg,u} \simeq -3.11$ ,  $e_0^b \tilde{c}_{1,\infty}^{rg,u} \simeq -0.305$ , respectively. We finally plot our corresponding rough evaluations of the two different time scales involved, too, with  $\tau_s$  describing the substrate decay time and  $\tau_c$  the complex saturation time. Notice that the time is in logarithmic scale.

Writing explicitly the contribution of the different terms, we obtain the following SPDERG 1st order UAs to the correct solutions for the time behaviour of the adimensional substrate concentration and of the adimensional complex concentration in MM kinetics beyond the sQSSA, respectively (with  $\omega$  the Lambert function):

$$\left\{ \begin{array}{l} \tilde{s}_1^{rg,u}(t) = M\omega(e^{-(M-m)t/M} + 1/M/M) + \\ \quad - \varepsilon \frac{1+m}{(1+M)^2} \left[ 1 - e^{-(1+M)t/\varepsilon} \right] + O(\varepsilon) \\ \tilde{c}_1^{rg,u}(t) = \frac{\omega(e^{-(M-m)t/M} + 1/M/M)}{\omega(e^{-(M-m)t/M} + 1/M/M) + 1} - \frac{1}{1+M} e^{-(1+M)t/\varepsilon} + \\ \quad - \varepsilon \frac{M(1+2m-M)}{(1+M)^4} \left[ 1 - e^{-(1+M)t/\varepsilon} \right] + \\ \quad - \varepsilon \left[ \frac{(1-M)(1+m)}{(1+M)^3} (t/\varepsilon) + \frac{M-m}{(1+M)^2} \frac{(t/\varepsilon)^2}{2} \right] e^{-(1+M)t/\varepsilon} + \\ \quad + \varepsilon \frac{(1+m)}{(1+M)^4} e^{-(1+M)t/\varepsilon} \left[ 1 - e^{-(1+M)t/\varepsilon} \right] + O(\varepsilon). \end{array} \right. \quad (44)$$

We plot in [Fig. 5] our corresponding results for the two considered sets of ICVs, as usual in comparison with the numerical solutions of the original problem (2) (the same curves as in [Fig. 1]).

The figure shows that, as expected, these results are more refined than the PE 0th order UA ([Fig. 2A], [Fig. 3A] and [Fig. 2C], [Fig. 3B] for the results for the  $a$  and  $b$  sets of ICVs, respectively), but not as much refined as the PE 1st order ones ([Fig. 4]). Moreover, the figure also makes evident

that, differently from the PE results, here the approximation fails in particular in the large time region. Actually, as expected, both the substrate and the complex approach (physically meaningless)  $O(\varepsilon)$  values for  $t \rightarrow \infty$ . In fact, from (44), one has  $\lim_{t \rightarrow \infty} \tilde{s}_1^{rg,u}(t) = \tilde{s}_{1,\infty}^{rg,u} = -\varepsilon(1+m)/(1+M)^2$  and  $\lim_{t \rightarrow \infty} \tilde{c}_1^{rg,u}(t) = \tilde{c}_{1,\infty}^{rg,u} = -\varepsilon M(1+2m-M)/(1+M)^4$  (the corresponding asymptotic constants for the two considered sets of ICVs are also plotted in the figures). Both this unphysical outcome and the lack of the 1st order contribution to the outer solution are particularly evident in the result for the substrate in the case of the  $b$  set of ICVs, corresponding to the larger  $\varepsilon = \varepsilon^b = 0.5$  ([Fig. 5C]).

On the other hand, noticeably, the figure definitely shows that the 1st order SPDERG approach works better than the 0th order PE method in capturing the qualitative features of the correct solutions. In detail, one can observe the presence of the three inflection points in the curves for the substrate both in [Fig. 5A] and in [Fig. 5C]. From the quantitative point of view, also the correct complex maximum values turn out to be better predicted than in the corresponding [Fig. 3], further confirming that the approach is particularly successful in the proximity of the matching region.

Therefore, on the basis of these initial results, that appear as a whole to support the SPDERG usefulness, both for further testing its correctness and for possibly obtaining better approximations to the correct solutions than the ones given by the PE 1st order UAs, we also consider the 2nd order in  $\varepsilon$ .

## 6 Results and discussion: ii) Second order contribution

In order to simplify the calculations, here we only look at the case in which the complex ICVs, in  $\tau = \tau_0$ , are fixed at  $\tilde{c}_0^* = \tilde{c}_1^* = \tilde{c}_2^* = 0$  from the beginning,  $\forall \tau_0$ . This could appear unrealistic, but it is not expected to influence the result, since we already checked that there is no need for renormalizing these bare constants at the 1st order, and the same thing should reasonably apply to the 2nd order, too.

From a different point of view, this choice is not expected to influence the results since at the end one is interested in taking as initial time  $\tau_0 = 0$ , and at the 2nd order, as at the 1st one, the renormalized part of  $\tilde{c}^{rg}$  should be obtainable by simply using the renormalized ICV for the substrate (after appropriately redefining the corresponding bare quantities for removing the secular terms). We will anyway verify in the following that indeed one gets the same ODE to be obeyed by  $\tilde{s}_1^{rg*}$  both from the study of the substrate and from the study of the complex, as it can be once again better understood within the analogy with the matching in the PE, where there is freedom for fixing only one of the two conditions, whereas the other turns out to be consistently also satisfied.

We take moreover  $\tilde{s}_2^* = 0$  from the beginning, since we expect that it can be anyway neglected within this approach at the 2nd order, in the same way as  $\tilde{s}_1^*$  turned out to be negligible at the 1st order (notice that we should instead allow for  $\tilde{s}_2^* \neq 0$  if we were to use these solutions to calculate the 3rd order contribution, too).

Clearly, one looks for 2nd order solutions in the form:

$$\begin{cases} \tilde{s}^{rg}(\tau) = \tilde{s}_0^{rg}(\tau) + \varepsilon \tilde{s}_1^{rg}(\tau) + \varepsilon^2 \tilde{s}_2^{rg}(\tau) \\ \tilde{c}^{rg}(\tau) = \tilde{c}_0^{rg}(\tau) + \varepsilon \tilde{c}_1^{rg}(\tau) + \varepsilon^2 \tilde{c}_2^{rg}(\tau). \end{cases} \quad (45)$$

Here,  $\tilde{s}_0^{rg}(\tau)$  and  $\tilde{c}_0^{rg}(\tau)$  are still given by (30), with  $\tilde{c}_0^* = 0$  in the case of the complex. Moreover,  $\tilde{s}_1^{rg}(\tau)$  and  $\tilde{c}_1^{rg}(\tau)$  are still given by (32) and (62), respectively, with  $\tilde{c}_1^* = 0$  in the case of the complex, whereas the condition  $\tilde{c}_0^* = 0$  does also apply to the solution for the substrate.

Correspondingly, we end up to study the system:

$$\begin{cases} \dot{\tilde{s}}_2^{rg}(\tau) &= (\tilde{s}_0^* + m)\tilde{c}_1^{rg}(\tau) + [\tilde{c}_0^{rg}(\tau) - 1]\tilde{s}_1^{rg}(\tau) \\ \dot{\tilde{c}}_2^{rg}(\tau) &= -(\tilde{s}_0^* + M)\tilde{c}_2^{rg}(\tau) - [\tilde{c}_0^{rg}(\tau) - 1]\tilde{s}_2^{rg}(\tau) - \tilde{s}_1^{rg}(\tau)\tilde{c}_1^{rg}(\tau), \end{cases} \quad (46)$$

whose solutions,  $\tilde{s}_2^{rg}(\tau)$  and  $\tilde{c}_2^{rg}(\tau)$ , with ICVs, at  $\tau = \tau_0$ ,  $\tilde{s}_2^{rg}(\tau_0) = 0$  and  $\tilde{c}_2^{rg}(\tau_0) = 0$ , are given in Appendix B.

The obtained formulas are anyway cumbersome, as one could expect: in fact, they contain both (implicitly) the 1st order contributions to the outer solutions and (explicitly) the 2nd order contributions to the inner ones. As anticipated, these last 2nd order contributions are calculated in the present work for the first time to our knowledge.

By carrying on the study as in the previous section, we start from the part of the substrate solution to be renormalized. Interestingly, beyond the 2nd order contribution that originates from the first three

terms in the solution for  $\tilde{s}_2^{rg}(\tau)$  given by (64) (that are the secular ones, whose coefficients are reported in (65)), the correct function needs also to contain the constant term that appears in  $s_1^{rg}(\tau)$  (32), since this is now a leading order term. In fact, it depends on one of the bare constants ( $\tilde{s}_0^*$ ), and it does not tend to zero for  $(\tau - \tau_0) \rightarrow \infty$ .

Hence, one finds:

$$\begin{aligned}\tilde{s}_{div}^{rg}(\tau) &= \tilde{s}_0^* + \varepsilon \tilde{s}_1^* - \varepsilon \frac{\tilde{s}_0^* + m}{(\tilde{s}_0^* + M)^2} \tilde{s}_0^* - \varepsilon \frac{(M - m) \tilde{s}_0^*}{\tilde{s}_0^* + M} (\tau - \tau_0) + \\ &+ \varepsilon^2 \frac{2M(M - m) \tilde{s}_0^*}{(\tilde{s}_0^* + M)^4} (\tilde{s}_0^* + m) (\tau - \tau_0) - \varepsilon^2 \frac{M(M - m) \tilde{s}_1^*}{(\tilde{s}_0^* + M)^2} (\tau - \tau_0) + \\ &+ \varepsilon^2 \frac{M(M - m)^2 \tilde{s}_0^*}{2(\tilde{s}_0^* + M)^3} (\tau - \tau_0)^2.\end{aligned}\quad (47)$$

We then replace the bare constants with the renormalized ones, *i.e.*,  $\tilde{s}_0^* = Z_{s_0}^s(\tau_0, \lambda) \tilde{s}_0^{rg*}(\lambda)$  and  $\tilde{s}_1^* = Z_{s_1}^s(\tau_0, \lambda) \tilde{s}_1^{rg*}(\lambda)$ , with, at the 2nd order in  $\varepsilon$ :

$$\begin{cases} Z_{s_0}(\tau_0, \lambda) &= 1 + \varepsilon z_{s_0,1}(\tau_0, \lambda) + \varepsilon^2 z_{s_0,2}(\tau_0, \lambda), \\ Z_{s_1}(\tau_0, \lambda) &= 1 + \varepsilon z_{s_1,1}(\tau_0, \lambda). \end{cases}\quad (48)$$

Correspondingly, we write  $(\tau - \tau_0) = (\tau - \lambda) + (\lambda - \tau_0)$  in (47) and we absorb the secular terms in  $(\lambda - \tau_0)$  by appropriately choosing the renormalization constant coefficients. In detail,  $z_{s_0,1}(\tau_0, \lambda)$  is already fixed by (35). Nevertheless, the contributions proportional to this coefficient to the third term and to the fourth one in (47) are to be taken carefully into account, since they turn out to be  $O(\varepsilon^2)$ .

In particular, in the case of the third term, when renormalizing  $\tilde{s}_0^*$ , one has:

$$-\varepsilon \frac{(\tilde{s}_0^* + m) \tilde{s}_0^*}{(\tilde{s}_0^* + M)^2} = -\varepsilon \frac{(\tilde{s}_0^{rg*} + m) \tilde{s}_0^{rg*}}{(\tilde{s}_0^{rg*} + M)^2} - \varepsilon^2 \left\{ \frac{d}{d\tilde{s}_0^*} \left[ \frac{(\tilde{s}_0^* + m) \tilde{s}_0^*}{(\tilde{s}_0^* + M)^2} \right] \Big|_{\tilde{s}_0^* = \tilde{s}_0^{rg*}} \right\} z_{s_0,1}(\tau_0, \lambda) \tilde{s}_0^{rg*}, \quad (49)$$

with:

$$\begin{aligned}& -\varepsilon^2 \left\{ \frac{d}{d\tilde{s}_0^*} \left[ \frac{(\tilde{s}_0^* + m) \tilde{s}_0^*}{(\tilde{s}_0^* + M)^2} \right] \Big|_{\tilde{s}_0^* = \tilde{s}_0^{rg*}} \right\} z_{s_0,1}(\tau_0, \lambda) \tilde{s}_0^{rg*} = \\ &= -\varepsilon^2 \left[ \frac{(M - m) \tilde{s}_0^{rg*}}{(\tilde{s}_0^{rg*} + M)^3} + \frac{M(\tilde{s}_0^{rg*} + m)}{(\tilde{s}_0^{rg*} + M)^3} \right] z_{s_0,1}(\tau_0, \lambda) \tilde{s}_0^{rg*} = \\ &= -\varepsilon^2 \left[ \frac{(M - m)^2 (\tilde{s}_0^{rg*})^2}{(\tilde{s}_0^{rg*} + M)^4} + \frac{M(M - m) \tilde{s}_0^{rg*}}{(\tilde{s}_0^{rg*} + M)^4} (\tilde{s}_0^{rg*} + m) \right] (\lambda - \tau_0).\end{aligned}\quad (50)$$

Clearly, both of these two terms do in fact contribute, and they have to be taken into account in the definition of  $z_{s_0,2}(\tau_0, \lambda)$ . Let us note moreover that  $d[\tilde{s}_0^*/(\tilde{s}_0^* + M)]/d\tilde{s}_0^* = M/(\tilde{s}_0^* + M)^2$ . Thus, one can check that the 2nd order contribution proportional to the same coefficient,  $z_{s_0,1}(\tau_0, \lambda)$  (that originates from the fourth term in  $\tilde{s}_{div}^{rg}$ , in the same way as we just explained in detail with the formulas (49) and (50)), partially absorbs the term proportional to  $(\tau - \lambda)(\lambda - \tau_0)$  (that originates from the last term), and partially contributes to  $z_{s_0,2}(\tau_0, \lambda)$ .

Thus, by taking:

$$\begin{cases} z_{s_0,2}(\tau_0, \lambda) &= \left[ \frac{(M - m)^2 \tilde{s}_0^{rg*}}{(\tilde{s}_0^{rg*} + M)^4} - \frac{M(M - m)}{(\tilde{s}_0^{rg*} + M)^4} (\tilde{s}_0^{rg*} + m) \right] (\lambda - \tau_0) + \\ &+ \frac{M(M - m)^2}{2(\tilde{s}_0^{rg*} + M)^3} (\lambda - \tau_0)^2 \\ z_{s_1,1}(\tau_0, \lambda) &= \frac{M(M - m)}{(\tilde{s}_0^{rg*} + M)^2} (\lambda - \tau_0), \end{cases}\quad (51)$$

we end up with an expression for  $\tilde{s}_{div}^{rg}(\tau, \lambda)$  that is exactly the same as the one given in (47), with  $\tilde{s}_0^* \rightarrow \tilde{s}_0^{rg*}(\lambda)$ ,  $\tilde{s}_1^* \rightarrow \tilde{s}_1^{rg*}(\lambda)$  and  $\tau_0 \rightarrow \lambda$ . Noticeably, moreover, though we wrote explicitly the first two terms in  $z_{s_0,2}$  to make evident the different origins of the contributions, their sum can be clearly simplified in such a way that the coefficient of the term proportional to  $(\lambda - \tau_0)$  is simply equal to  $-m(M - m)/(\tilde{s}_0^{rg*} + M)^3$ .

Therefore, we continue our analysis by studying directly the derivative with respect to  $\lambda$  of  $\tilde{s}_{div}^{rg}(\tau, \lambda)$ , and we obtain, at the 2nd order:

$$\begin{aligned}
\frac{d\tilde{s}_{div}^{rg}(\tau, \lambda)}{d\lambda} &= \frac{d\tilde{s}_0^{rg*}(\lambda)}{d\lambda} + \varepsilon \frac{d\tilde{s}_1^{rg*}(\lambda)}{d\lambda} + \varepsilon \frac{(M-m)\tilde{s}_0^{rg*}(\lambda)}{\tilde{s}_0^{rg*}(\lambda) + M} + \varepsilon^2 \frac{(M-m)^2 [\tilde{s}_0^{rg*}(\lambda)]^2}{[\tilde{s}_0^{rg*}(\lambda) + M]^4} + \\
&+ \varepsilon^2 \frac{M(M-m)\tilde{s}_0^{rg*}(\lambda)}{[\tilde{s}_0^{rg*}(\lambda) + M]^4} [\tilde{s}_0^{rg*}(\lambda) + m] + \varepsilon^2 \frac{M(M-m)^2 \tilde{s}_0^{rg*}(\lambda)}{[\tilde{s}_0^{rg*}(\lambda) + M]^3} (\tau - \lambda) + \\
&- \varepsilon^2 \frac{2M(M-m)\tilde{s}_0^{rg*}(\lambda)}{[\tilde{s}_0^{rg*}(\lambda) + M]^4} [\tilde{s}_0^{rg*}(\lambda) + m] + \varepsilon^2 \frac{M(M-m)\tilde{s}_1^{rg*}(\lambda)}{[\tilde{s}_0^{rg*}(\lambda) + M]^2} + \\
&- \varepsilon^2 \frac{M(M-m)^2 \tilde{s}_0^{rg*}(\lambda)}{[\tilde{s}_0^{rg*}(\lambda) + M]^3} (\tau - \lambda). \tag{52}
\end{aligned}$$

In fact, one already makes use of the known 1st order result on  $d\tilde{s}_0^{rg*}/d\lambda$ , given in Eqs. (38), in the derivation of this equation.

Here, for the sake of clarity, we also wrote explicitly both of the 2nd order terms proportional to  $(\tau - \lambda)$ , that obviously cancel each other. In fact, this appears a quite consistent result of the present approach, since they have completely different origins. Indeed, the first of these terms originates from part of the derivative with respect to  $\lambda$  of the 1st order term proportional to  $(\tau - \lambda)$  in  $\tilde{s}_{div}^{rg}(\tau, \lambda)$  (*i.e.*, from the term that is equal to  $-\varepsilon(M-m)\{d[\tilde{s}_0^{rg*}/(\tilde{s}_0^{rg*} + M)]/d\lambda\}(\tau - \lambda)$ ). The second of these terms originates instead from the derivative with respect to  $\lambda$  of the last term in  $\tilde{s}_{div}^{rg}(\tau, \lambda)$ , that is proportional to  $(\tau - \lambda)^2$ .

Moreover, interestingly, the second term proportional to  $\varepsilon^2$  (part of the contribution that originates from the constant term in  $\tilde{s}_1^{rg}(\tau)$ ), letting aside a factor 2, is the same as the one that is found when deriving the first of the terms proportional to  $\varepsilon^2(\tau - \lambda)$  in (47) with respect to  $\lambda$  (*i.e.*, the term in  $\tilde{s}_{div}^{rg}(\tau, \lambda)$  that corresponds to the fourth term proportional to  $\varepsilon^2$  here), but that has opposite sign.

In fact, both of these results are obtained in a similar manner to the one that we described in detail previously, in the context of the derivation of the appropriate renormalization constants (from Eqs. (38), one has  $(\lambda - \tau_0)d\tilde{s}_0^{rg*}/d\lambda = -\varepsilon z_{s_0,1}(\tau_0, \lambda)\tilde{s}_0^{rg*}$ ).

Finally, by imposing the scaling condition  $d\tilde{s}_{div}^{rg}(\tau, \lambda)/d\lambda = 0$ , and by grouping the terms in  $\varepsilon$  and in  $\varepsilon^2$ , we derive the two ODEs to be obeyed by  $\tilde{s}_0^{rg*}(\lambda)$  and  $\tilde{s}_1^{rg*}(\lambda)$ , respectively:

$$\left\{ \begin{aligned} \frac{d\tilde{s}_0^{rg*}(\lambda)}{d\lambda} &= -\varepsilon \frac{(M-m)\tilde{s}_0^{rg*}(\lambda)}{\tilde{s}_0^{rg*}(\lambda) + M} \\ \frac{d\tilde{s}_1^{rg*}(\lambda)}{d\lambda} &= \varepsilon \left\{ -\frac{(M-m)^2 [\tilde{s}_0^{rg*}(\lambda)]^2}{[\tilde{s}_0^{rg*}(\lambda) + M]^4} + \frac{M(M-m)\tilde{s}_0^{rg*}(\lambda)}{[\tilde{s}_0^{rg*}(\lambda) + M]^4} [\tilde{s}_0^{rg*}(\lambda) + m] + \right. \\ &\quad \left. - \frac{M(M-m)\tilde{s}_1^{rg*}(\lambda)}{[\tilde{s}_0^{rg*}(\lambda) + M]^2} \right\} = \varepsilon \left\{ \frac{m(M-m)\tilde{s}_0^{rg*}(\lambda)}{[\tilde{s}_0^{rg*}(\lambda) + M]^3} - \frac{M(M-m)\tilde{s}_1^{rg*}(\lambda)}{[\tilde{s}_0^{rg*}(\lambda) + M]^2} \right\}. \end{aligned} \right. \tag{53}$$

The first of these ODEs is just the already known 1st order result for  $d\tilde{s}_0^{rg*}(\lambda)/d\lambda$  reported in Eqs. (38). Actually, at the 2nd order, the interesting ODE is the one to be obeyed by  $\tilde{s}_1^{rg*}(\lambda)$ .

Let us remark that, when making the final transformation  $\lambda \rightarrow \tau = t/\varepsilon$ , and when recalling that  $\tilde{s}_0^{rg*}(t) = \tilde{s}_0^{out}(t)$  (given in (8)), the ODE to be obeyed by  $\tilde{s}_1^{rg*}(t)$  turns out to be different from the one for the 1st order outer substrate within the PE method reported in Eqs. (19). Here, we wrote the equation both with and without the simplification due to the sum of the first two terms just in order to make evident the difference, since it is the first term in the not simplified expression that was absent there. Indeed, to get in particular the same coefficient as in the ODE in Eqs. (19) for the second term in the present not simplified expression, it is essential to correctly take into account the constant in  $\tilde{s}_1^{rg}(\tau)$  in the part to be renormalized of the function (at the 2nd order). In fact, as outlined, the contribution of the only term proportional to  $\varepsilon^2$  would give an incorrect (twice larger) coefficient.

Interestingly, the present ODE turns out to be simpler than the one encountered in the PE method. One can check that the solution is given by:

$$\tilde{s}_1^{rg*}(t) = \frac{m\tilde{s}_0^{out}(t)}{M[\tilde{s}_0^{out}(t) + M]} \log \left[ \frac{\tilde{s}_0^{out}(t) + M}{(1 + M)\tilde{s}_0^{out}(t)} \right], \tag{54}$$



with the choice  $\tilde{s}_1^{rg*}(0) = 0$ , that is reasonable in this context, since it allows to get correctly  $\tilde{s}_2^{rg,u}(0) = 1$ . It is also important to stress that the solution also satisfies the asymptotic condition  $\lim_{t \rightarrow \infty} \tilde{s}_1^{rg*}(t) = 0$ , though this in fact implies  $\lim_{t \rightarrow \infty} \tilde{s}_2^{rg,u}(t) = O(\varepsilon^2)$ .

On the other hand, consistently, when adding the 1st order term that originates from the replacement  $\tilde{s}_0^* \rightarrow \tilde{s}_0^{rg,*}(\lambda)$  in the 1st order constant term in the part to be renormalized of the function, the total 1st order *outer* contribution to the SPDERG 2nd order UA to the correct solution for the substrate is just:

$$\tilde{s}_1^{rg,out}(t) = -\frac{[\tilde{s}_0^{out}(t) + m]\tilde{s}_0^{out}(t)}{[\tilde{s}_0^{out}(t) + M]^2} + \tilde{s}_1^{rg*}(t) = \tilde{s}_1^{out}(t). \quad (55)$$

Thus, one recovers the 1st order outer contribution to the PE UA reported in (20). As we will discuss more in detail in the following, this was not a result to be taken for granted [15]. Obviously, the result implies that the total 1st order outer contribution satisfies the MC, too (*i.e.*, in particular that  $\tilde{s}_1^{rg,out}(0) = -(1+m)/(1+M)^2$ ).

Hence, we recall that  $\mathcal{R}_2^s(\tau)$  is to be evaluated from the remaining part of the solution in  $\tilde{s}_0^* = 1$ ,  $\tilde{s}_1^* = 0$  and  $\tau_0 = 0$ . It is given by (see (64) and (66), in Appendix B):

$$\begin{aligned} \mathcal{R}_2^s(\tau) &= D_{s_2^{rg}}(1) + \left[ F_{s_2^{rg}}(1) + H_{s_2^{rg}}(1)\tau + J_{s_2^{rg}}(1)\tau^2 \right] e^{-(1+M)\tau} + K_{s_2^{rg}}(1)e^{-2(1+M)\tau} = \\ &= -\frac{1}{2(1+M)^5} [2M^2(2m+1) - M(6m^2 + 5m + 3) - m^2 + m] + \\ &+ \frac{1}{(1+M)^5} [M^2(2m+1) - M(3m^2 + 3m + 2) - m^2 - m - 1] e^{-(1+M)\tau} + \\ &- \left\{ \frac{1}{(1+M)^4} [M^2 + M(m^2 + 1) - 2m - 1]\tau - \frac{(M-m)}{2(1+M)^3} (m+1)\tau^2 \right\} e^{-(1+M)\tau} + \\ &+ \frac{(m+1)}{2(1+M)^5} (M+m+2) e^{-2(1+M)\tau}, \end{aligned} \quad (56)$$

by using in particular the coefficient values reported in (66).

Correspondingly, we get the complete result for the SPDERG 2nd order UA to the adimensional substrate solution:

$$\tilde{s}_2^{rg,u}(t) = \tilde{s}_1^{rg,u}(t) + \varepsilon \left\{ -\frac{\tilde{s}_0^{out}(t) + m}{[\tilde{s}_0^{out}(t) + M]^2} \tilde{s}_0^{out}(t) + \frac{1+m}{(1+M)^2} + \tilde{s}_1^{rg*}(t) \right\} + \varepsilon^2 \mathcal{R}_2^s(t/\varepsilon) + O(\varepsilon^2), \quad (57)$$

with  $\tilde{s}_1^{rg,u}(t)$  given in (44),  $\tilde{s}_0^{out}(t) = \tilde{s}_0^{rg*}(t)$  given in (8), and  $\tilde{s}_1^{rg*}(t)$ ,  $\mathcal{R}_2^s(t/\varepsilon)$ , given in (54), (56), respectively. We wrote the solution in the present form to make more evident the analogies and the differences with the 1st order PE result. In particular, the constant term that is equal to  $-(1+m)/(1+M)^2$  originates here from the constant term depending on  $\tilde{s}_0^*$  that appeared (calculated in  $\tilde{s}_0^* = 1$ ) in  $\tilde{s}_1^{rg,u}(t)$ , the one that has been now included in  $\tilde{s}_{div}^{rg}$  at the 2nd order. Moreover, one can notice that the result implies, as expected,  $\lim_{t \rightarrow \infty} \tilde{s}_2^{rg,u}(t) = \tilde{s}_{2,\infty}^{rg,u} = \varepsilon^2 D_{s_2^{rg}}(1) = O(\varepsilon^2)$ , where  $D_{s_2^{rg}}(1)$  is the first of the terms in (56), reported in Appendix B, too.

For the complex, the 2nd order terms to be added to the part of the solution to be renormalized are the three ones that appear in  $\tilde{c}_{2,div}^{rg}(\tau)$ , that is reported in (68), in Appendix A. Moreover, we have again to take into account also the constant term in  $\tilde{c}_1^{rg}(\tau)$ , *i.e.*, the one corresponding to the coefficient  $B_{c_1^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*, \tilde{c}_0^*)$  that is reported in (63), in Appendix A, and that needs to be calculated in  $\tilde{c}_0^* = 0$ . We obtain:

$$\begin{aligned} \tilde{c}_{div}^{rg}(\tau) &= \frac{\tilde{s}_0^*}{\tilde{s}_0^* + M} - \varepsilon \frac{M(\tilde{s}_0^* - M + 2m)}{(\tilde{s}_0^* + M)^4} \tilde{s}_0^* + \varepsilon \frac{M\tilde{s}_1^*}{(\tilde{s}_0^* + M)^2} - \varepsilon \frac{M(M-m)\tilde{s}_0^*}{(\tilde{s}_0^* + M)^3} (\tau - \tau_0) + \\ &+ \varepsilon^2 A_{c_{2,div}^{rg}}(\tilde{s}_0^*)(\tau - \tau_0) + \varepsilon^2 B_{c_{2,div}^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*)(\tau - \tau_0) + \varepsilon^2 C_{c_{2,div}^{rg}}(\tilde{s}_0^*)(\tau - \tau_0)^2, \end{aligned} \quad (58)$$

where the coefficients  $A_{c_{2,div}^{rg}}$ ,  $B_{c_{2,div}^{rg}}$  and  $C_{c_{2,div}^{rg}}$  of the three 2nd order secular terms are given in (69), in Appendix B.

Though the proof involves more demanding calculations in the present case, one can check that here, in the same way as we showed in detail in the case of the substrate, with the same renormalization coefficients of the ICVs ( $z_{s0,1}$  already fixed in (35), and  $z_{s0,2}$ ,  $z_{s1,1}$  already fixed in (51), respectively), one obtains a function  $\tilde{c}_{div}^{rg}(\tau, \lambda)$  exactly corresponding to (58), with  $\tilde{s}_0^* \rightarrow \tilde{s}_0^{rg*}(\lambda)$ ,  $\tilde{s}_1^* \rightarrow \tilde{s}_1^{rg*}(\lambda)$  and

$\tau_0 \rightarrow \lambda$ . This was in fact the expected result from the point of view of the analogy with the matching in the PE.

Moreover, as furthermore expected within the same context, we verify that, when imposing the scaling condition  $d\tilde{c}_{div}^{rg}(\tau, \lambda)/d\lambda = 0$ , one recovers once again the two ODEs in (53), to be obeyed by  $\tilde{s}_0^{rg*}(\lambda)$  and  $\tilde{s}_1^{rg*}(\lambda)$ . Thus, one obtains in particular the same result on  $\tilde{s}_1^{rg*}(\lambda)$ .

In detail, also in the case of the complex, one needs to use the known 1st order result on  $d\tilde{s}_0^{rg*}(\lambda)/d\lambda$  in the derivation of the 2nd order one on  $d\tilde{s}_1^{rg*}(\lambda)/d\lambda$ . Finally, also in this case the result on the derivative with respect to  $\lambda$  is obtained in a very similar way to the proof of the correspondence between  $\tilde{c}_{div}^{rg}(\tau, \lambda)$  and the original  $\tilde{c}_{div}^{rg}(\tau)$  given by (58).

Clearly, in the present case, there is a definitely larger number of relevant terms that anyway either cancel each other or contribute in the correct way to the final result. Therefore, the verification of the expectations we made on the basis of the recalled analogy, that is reported in Appendix C, appears to give further consistency to the whole approach.

Correspondingly, we obtain a 1st order *outer* contribution to the SPDERG 2nd order UA to the correct solutions for the adimensional complex concentration that turns out to be exactly equal to the 1st order PE outer contribution given in (21). It is obtained here from an algebraic relation that could appear different from the one in Eqs. (19), but that is in fact equivalent. This becomes evident when writing  $\tilde{s}_1^{rg*}(t)$  in terms of  $\tilde{s}_0^{out}(t)$  and  $\tilde{s}_1^{out}(t)$  by means of (55):

$$\tilde{c}_1^{rg,out}(t) = -\frac{M(\tilde{s}_0^{rg*}(t) - M + 2m)}{[\tilde{s}_0^{rg*}(t) + M]^4} \tilde{s}_0^{rg*}(t) + \frac{M\tilde{s}_1^{rg*}(t)}{[\tilde{s}_0^{rg*}(t) + M]^2} = \tilde{c}_1^{out}(t). \quad (59)$$

The equality can also be easily checked by reminding that  $\tilde{s}_0^{rg*}(t) = \tilde{s}_0^{out}(t)$ , and by using the known result for  $\tilde{s}_1^{rg*}(t)$  reported in (54).

The SPDERG 2nd order UA to the correct solution for the complex is then obtainable by taking into account also the remaining part of the 2nd order inner solution, and is given by:

$$\begin{aligned} \tilde{c}_2^{rg,u}(t) &= \tilde{c}_1^{rg,u}(t) + \varepsilon \left\{ -\frac{M(\tilde{s}_0^{rg*}(t) - M + 2m)}{[\tilde{s}_0^{rg*}(t) + M]^4} \tilde{s}_0^{rg*}(t) + \frac{M\tilde{s}_1^{rg*}(t)}{[\tilde{s}_0^{rg*}(t) + M]^2} + \frac{M(1 - M + 2m)}{(1 + M)^4} \right\} + \\ &+ \varepsilon^2 \mathcal{R}_2^c(t/\varepsilon) + O(\varepsilon^2). \end{aligned} \quad (60)$$

Here, we outline once again similarities and differences with the PE result. In fact, because of (59), one can equivalently write the terms in curly brackets as  $\tilde{c}_1^{out}(t)$  minus the constant terms that in the standard method appear twice.

In detail, the various terms that appear in (60) are reported:  $\tilde{c}_1^{rg,u}(t)$  in (44);  $\tilde{s}_0^{rg*}(t) = \tilde{s}_0^{out}(t)$  in (8);  $\tilde{s}_1^{rg*}(t)$  in (54); and  $\mathcal{R}_2^c(\tau)$ , correctly evaluated in  $\tau_0 = 0$ ,  $\tilde{s}_0^* = 1$  and  $\tilde{s}_1^* = 0$  in (70), with the coefficients given in (72), in Appendix B.

This UA to the correct solution verifies the ICV  $\tilde{c}_2^{rg,u}(0) = 0$ , whereas one finds  $\lim_{t \rightarrow \infty} \tilde{c}_2^{rg,u}(t) = \tilde{c}_{2,\infty}^{rg,u} = \varepsilon^2 A_{\mathcal{R}_2^c}(1, 0) = O(\varepsilon^2)$ , with  $A_{\mathcal{R}_2^c}(1, 0)$  the constant term in  $\mathcal{R}_2^c(\tau)$ , whose detailed dependence on  $\tilde{s}_0^*$  and  $\tilde{s}_1^*$  is reported in (71), and that is calculated in  $\tilde{s}_0^* = 1$ ,  $\tilde{s}_1^* = 0$  in (72), in Appendix B.

We plot in [Fig. 6] our numerical results on the SPDERG 2nd order UAs to the solutions for the substrate and the complex, respectively, for the two considered sets of ICVs. The plots are as usual in comparison with the numerical solutions of the original problem (2) (the same curves as in [Fig. 1]).

As it is in fact explicable on the basis that they contain the 2nd order terms of the inner solutions, the approximations work better than the 1st order PE ones in a region that encompasses the matching one. Indeed, the results here are nearly indistinguishable, within our numerical precision, from the correct ones on a definitely larger time window. This is true also in the particularly demanding case of the substrate in [Fig. 6C], and the outcome is clearly different from the one observed in the same case at the 1st order, when applying the standard method, that is reported in [Fig. 4C].

Nevertheless, one can still note a minor discrepancy at large times, that is at least partially to be related to the failure of the approximations in reproducing the physically correct asymptotically vanishing solutions. Actually, on the basis of the results that we already obtained in the present study, this failure appears easily correctable in a reasonable way. In the following, we just consider the SPDERG 2nd order UAs that can be proposed that satisfy the asymptotic conditions  $\lim_{t \rightarrow \infty} s(t) = \lim_{t \rightarrow \infty} c(t) = 0$ , too.

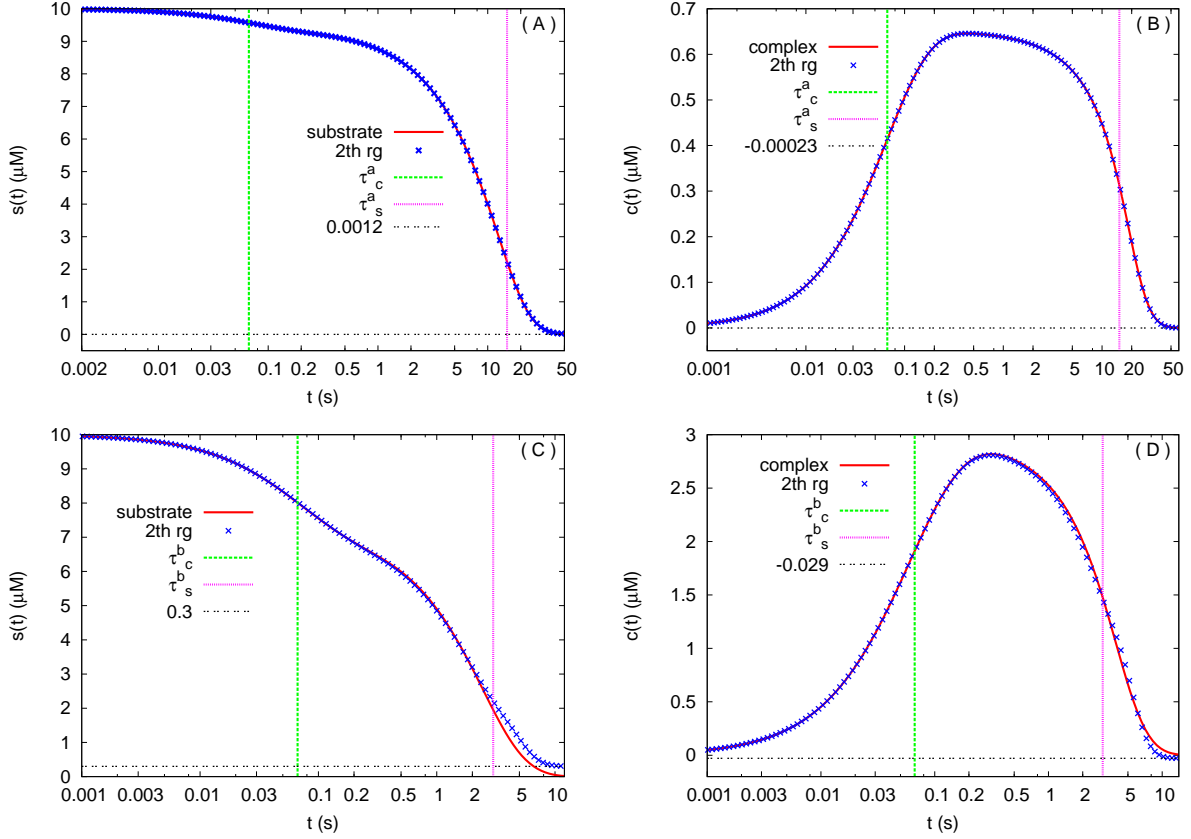


Figure 6: In A) and C) we present the behaviour of the concentrations of the substrate  $s(t)$ , whereas in B) and D) we present the ones of the complex  $c(t)$  for the  $a$  and  $b$  sets of ICVs given in (4), respectively. Hence, in A) and B) we are in the case with  $\varepsilon = \varepsilon^a = 0.1$ , whereas in C) and D) we are in the one with  $\varepsilon = \varepsilon^b = 0.5$ . We plot both the numerical solutions of Eqs. (2) already shown in the previous figures, and the analytical solutions computed from the SPDERG 2nd order UAs (with a standard numerical approximation for the Lambert function), as given in (57) and (60), respectively. We plot moreover the (physically meaningless) asymptotic limits of these analytical solutions:  $s_0^a \tilde{s}_{2,\infty}^{rg,u} \simeq 0.0012$ ,  $e_0^a \tilde{c}_{2,\infty}^{rg,u} \simeq -0.00023$ , and  $s_0^b \tilde{s}_{2,\infty}^{rg,u} \simeq 0.3$ ,  $e_0^b \tilde{c}_{2,\infty}^{rg,u} \simeq -0.029$ , for the  $a$  and  $b$  ICV sets, respectively. We finally plot our corresponding rough evaluations of the two different time scales involved, too, with  $\tau_s$  describing the substrate decay time and  $\tau_c$  the complex saturation time. Notice that the time is in logarithmic scale.

## 7 Results and discussion: iii) Second order with proposed refinement

Within the framework of the already obtained results, by assuming that the found solution behaviours could be iterated, one can hypothesize that, in the SPDERG approach (at least in its present kind of application to a boundary layer problem, in which we directly renormalized the bare initial constant values), the constant term at a given order will contribute to the outer component of the solution at the following order. Actually, this appears to us the SPDERG approach ingredient that is equivalent to take into account both the MCs and the removing of one of the constants that appear twice in the standard PE method, though the observation needs to be better formalized for investigating its possible generalizations.

In fact, in the present case, when passing from the 2nd to the 3rd order, these iteratively expected solution behaviours should be obtainable by means of the substitutions  $\tilde{s}_0^* \rightarrow \tilde{s}_0^{rg*}(t)$  and  $\tilde{s}_1^* \rightarrow \tilde{s}_1^{rg*}(t)$  in the constant terms  $D_{s_2^{rg}}(\tilde{s}_0^*) + E_{s_2^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*)$  and  $A_{\mathcal{R}_2^c}(\tilde{s}_0^*, \tilde{s}_1^*)$ . These terms appear in the 2nd order inner solution for the substrate and the complex, respectively, that are given in (66) and (71), in Appendix B. We notice that, despite of the substitutions, they will remain terms of 2nd order, in agreement with the general consideration that, at the  $n$ -th order, within the SPDERG approach, one in fact obtains the  $(n-1)$ -th order outer components of the corresponding PE UAs, to be interpreted as their leading order terms [14, 15].

Therefore, we can finally consider as refined SPDERG 2nd order UAs to the correct solutions the functions  $\tilde{s}_{2,r}^{rg,u}(t)$  and  $\tilde{c}_{2,r}^{rg,u}(t)$ , that satisfy by construction the physically meaningful asymptotic

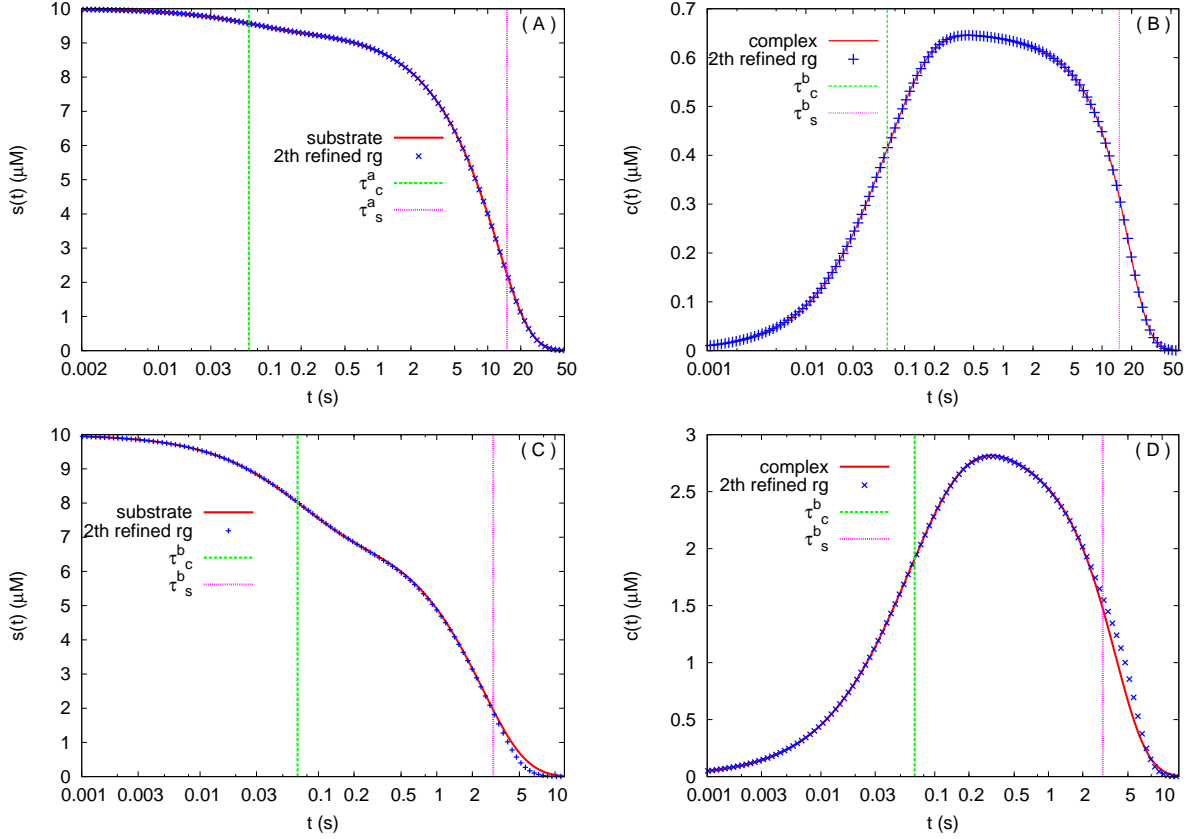


Figure 7: In A) and C) we present the behaviour of the concentrations of the substrate  $s(t)$ , whereas in B) and D) we present the ones of the complex  $c(t)$  for the  $a$  and  $b$  sets of ICVs given in (4), respectively. Hence, in A) and B) we are in the case with  $\varepsilon = \varepsilon^a = 0.1$ , whereas in C) and D) we are in the one with  $\varepsilon = \varepsilon^b = 0.5$ . We plot both the numerical solutions of Eqs. (2) already shown in the previous figures, and the analytical solutions computed from the refined SPDERG 2nd order UAs (with a standard numerical approximation for the Lambert function), as given in (61). We finally plot our corresponding rough evaluations of the two different time scales involved, too, with  $\tau_s$  describing the substrate decay time and  $\tau_c$  the complex saturation time. Notice that the time is in logarithmic scale.

conditions  $\lim_{t \rightarrow \infty} \tilde{s}_{2,r}^{rg,u}(t) = \lim_{t \rightarrow \infty} \tilde{c}_{2,r}^{rg,u}(t) = 0$ , given by:

$$\begin{cases} \tilde{s}_{2,r}^{rg,u}(t) &= \tilde{s}_2^{rg,u}(t) + \varepsilon^2 \left\{ D_{s_2^{rg}}[\tilde{s}_0^{rg*}(t)] + E_{s_2^{rg}}[\tilde{s}_0^{rg*}(t), \tilde{s}_1^{rg*}(t)] - D_{s_2^{rg}}(1) \right\} + O(\varepsilon^2) \\ \tilde{c}_{2,r}^{rg,u}(t) &= \tilde{c}_2^{rg,u}(t) + \varepsilon^2 \left\{ A_{\mathcal{R}_2^c}[\tilde{s}_0^{rg*}(t), \tilde{s}_1^{rg*}(t)] - A_{\mathcal{R}_2^c}(1, 0) \right\} + O(\varepsilon^2), \end{cases} \quad (61)$$

We remind that  $E_{s_2^{rg}}(1, 0) = 0$ , whereas  $\tilde{s}_2^{rg,u}(t)$  and  $\tilde{c}_2^{rg,u}(t)$  are reported in (57) and (60), respectively,  $\tilde{s}_0^{rg*}(t) = \tilde{s}_0^{out}(t)$  in (20), and  $\tilde{s}_1^{rg*}(t)$  in (54).

It is not to be taken for granted that these approximations could work better than the previously considered ones, since they anyway lack of a part of the 2nd order outer contribution. On the other hand, these appear to us the most refined SPDERG 2nd order UAs that one can propose by exploiting as much as possible the obtained results.

We present the corresponding numerical solutions for the two different considered ICV sets in [Fig. 7]. The plots are as usual in comparison with the numerical solutions of the original problem (2) (the same curves as in [Fig. 1]).

In fact, one could already observe in the previous [Fig. 6A], [Fig. 6B] that the SPDERG 2nd order UAs appeared indistinguishable from the correct solutions in the case of the  $a$  set of ICVs. Indeed, this set corresponds to the relatively small  $\varepsilon = \varepsilon^a = 0.1$ , and the PE 1st order UAs appeared indistinguishable from the correct solutions for this ICV set ([Fig. 4A], [Fig. 4B]), too. In this case, we limit ourselves to underline that the present proposed 2nd order approximations, that are presented in [Fig. 7A] for the substrate concentration and in [Fig. 7B] for the complex one, are moreover correctly asymptotically vanishing. In fact, as previously discussed, in the not refined 2nd order approximations the limits for  $t \rightarrow \infty$  were not zero, though they gave a practically negligible contribution to the plotted curves.

On the other hand, when looking at [Fig. 7C] and [Fig. 7D], that present the behaviour of the substrate and complex concentration, respectively, for the  $b$  ICV case, *i.e.*, for the larger value of the expansion parameter  $\varepsilon = \varepsilon^b = 0.5$ , the plots appear not enough detailed for understanding up to which point the present approximated solutions are better than the ones without the refinement. Both for this reason and for roughly quantifying the differences between the PE 1st order UAs and the present SPDERG 2nd order results, we are led to a more careful study.

## 8 Results and discussion: iv) A conclusive comparison

We present in [Fig. 8] the detailed time depending behaviours of the substrate concentration,  $s(t)$ , and of the complex one,  $c(t)$ , for the more demanding ICV case with  $\varepsilon = \varepsilon^b = 0.5$ , in the two relevant parts of the time window, by comparing the different *best* approximations we considered: i) the PE 1st order UAs (as given in (25), already presented in [Fig. 4C], [Fig. 4D]); ii) the SPDERG 2nd order UAs (as given in (57), (60), already presented in [Fig. 6C], [Fig. 6D]); iii) the refined SPDERG 2nd order UAs (as given in (61), already presented in [Fig. 7C], [Fig. 7D]).

Here, we neglect first of all the initial time interval, up to  $t = 0.03s$  for the substrate and to  $t = 0.08s$  for the complex, respectively. Indeed, in this interval, the different results are indistinguishable, within our numerical precision, both each other and with the correct numerical solutions. Instead, we consider in detail, as usual in logarithmic time scale, the central intervals, *i.e.*, the ones that encompass the matching region. These intervals are  $t \in [0.03, 3.5]s$  for the substrate and  $t \in [0.08, 2.5]s$  for the complex, respectively. Finally, we present in non logarithmic time scale the relevant large time window, [Fig. 8B] and [Fig. 8D], for the substrate and the complex, respectively. In fact, this window ranges up to  $t \sim 12s$  for the substrate and up to  $t \sim 14s$  for the complex, since at larger times (as it is clear from [Fig. 8B] in particular) the solutions have already reached, within our numerical precision, their asymptotic values (that are different from zero in the case of the non refined SPDERG).

Interestingly, the curves shown in [Fig. 8A] not only make once more evident that, in the case of the substrate, the SPDERG 2nd order UAs we considered works definitely better than the PE 1st order ones, both with and without the refinement, but it also allows to naked-eye evaluate the range in which this happens. In fact, this range corresponds to  $t \sim 0.05 \div 2s$ , hence it covers about the 15% of the whole relevant time window in the case without refinement, whereas it corresponds to  $t \sim 0.05 \div 3s$  (with the larger time being of the same order of  $\tau_s^b$ ), and hence it covers about the 25% of the whole relevant time window, in the case with the refinement. On the other hand, for  $t \gtrsim 3s$  ([Fig. 8B]), the refined SPDERG 2nd order UA appears to tend to zero slightly too rapidly, with respect both to the correct solution and to the PE 1st order result, though it makes a smaller error than the same SPDERG approximation without the refinement.

In the case of the complex, as already anticipated, the curves shown in [Fig. 8C] make clear that the PE 1st order UA slightly over-evaluates the value of the maximum of this quantity. In fact, it appears to over-evaluate the dynamical behaviour of this quantity in the whole range  $t \sim 0.25 \div 0.7s$ , that is a time interval roughly centered around the maximum abscissa. On the other hand, both of the SPDERG 2nd order UAs that we considered are successful in correctly capturing the maximum value of the complex numerical solution. In particular, the refined SPDERG approximation turns out to be the one more in agreement with the correct result up to the larger time  $t \sim 2s$ , *i.e.*, in about the 15% of the whole relevant time window. It is moreover both as much correct as the 1st order result of the standard method (though going to zero slightly more rapidly than the correct solution) and definitely better than the same approximation without refinement at large times. In particular, this is true for  $t \sim 9 \div 14s$ , that covers about a remaining 35% of the whole relevant time window. Nevertheless, the refined SPDERG approximation turns out to be the one that makes the largest error, by over-evaluating the correct complex concentration behaviour, in the remaining part of the relevant time window (in detail, for  $t \sim 2.5 \div 6.5s$ ).

Thus, the refined SPDERG 2nd order UAs we proposed successfully capture the correct dynamical behaviour in a large part of the relevant time window, despite of the considered case being very demanding. First of all, this further confirms both the correctness and the utility of the SPDERG approach in general. Moreover, these findings support the correctness of the present proposed way for obtaining asymptotically vanishing solutions, that exploits as much as possible the results, too. At least within our kind of SPDERG application procedure, the proposed refined SPDERG UAs appear easily generalizable to other similar cases. At the same time, the present analysis makes clear that the



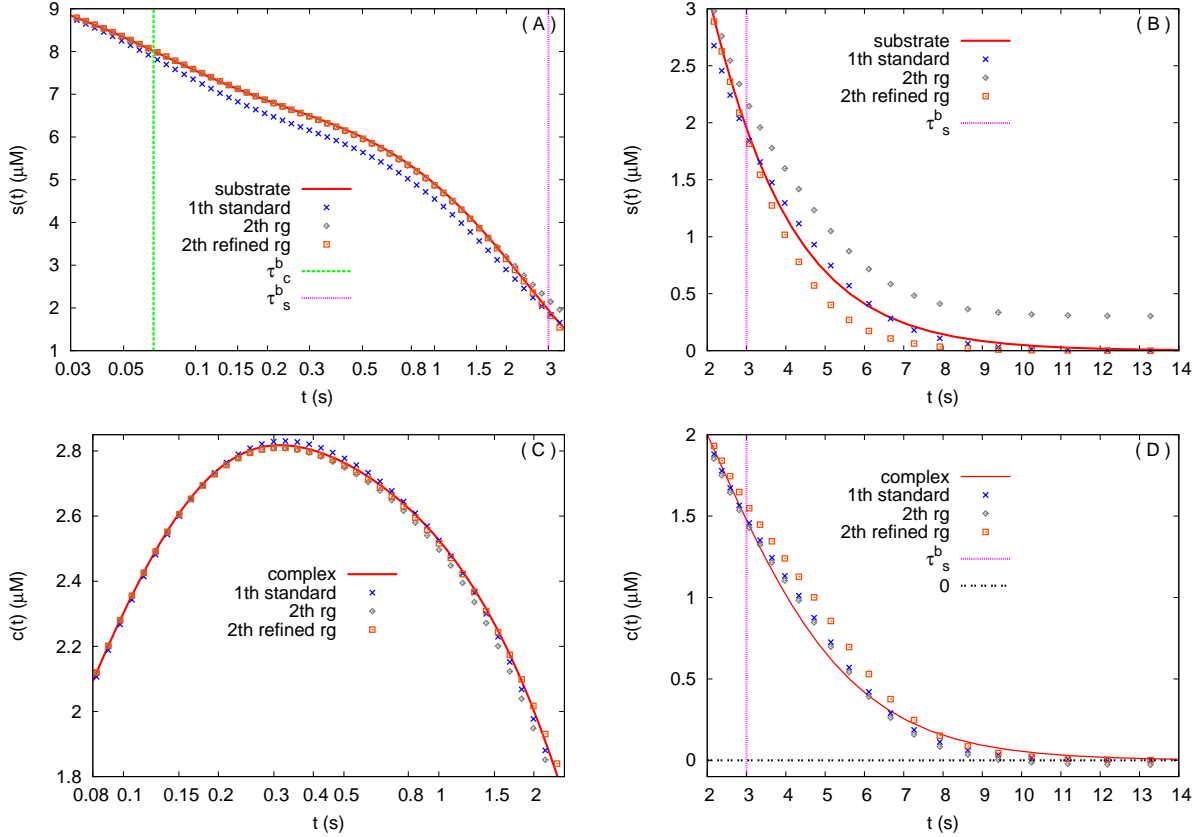


Figure 8: In A) (respectively C)) we present the behaviour of the concentration of the substrate  $s(t)$  (respectively the complex  $c(t)$ ) in the central part of the time window, in logarithmic time scale, whereas in B) (respectively D)) we present the large time behaviour of these two quantities. The results are for the  $b$  set of ICVs given in (4), *i.e.*, with  $\varepsilon = \varepsilon^b = 0.5$ . We plot the numerical solutions of Eqs. (2), already shown in the previous figures, and we compare with these correct behaviours: i) the PE 1st order UAs (as given in (25), already presented in [Fig. 4C], [Fig. 4D]); ii) the SPDERG 2nd order UAs (as given in (57), (60), already presented in [Fig. 6C], [Fig. 6D]); iii) the refined SPDERG 2nd order UAs (as given in (61), already presented in [Fig. 7C], [Fig. 7D]). All these UAs to the correct solutions are computed by means of the same standard numerical approximation for the Lambert function. When they belong to the relevant time window, we finally plot our corresponding rough evaluations of the two different time scales involved, too, with  $\tau_s^b$  describing the substrate decay time and  $\tau_c^b$  the complex saturation time.

remaining part, to be analytically calculated, of the 2nd order outer contributions would be important for an approximation to MM kinetics, beyond the sQSSA, that could work in the whole relevant time window, whatever the kinetic constants are, for values of the expansion parameter as large as  $\varepsilon = e_0/s_0 \sim 0.5$ .

## 9 Conclusions

In the present work, we start by recalling the standard PE method in the case of MM kinetics, beyond the sQSSA [2, 3, 4, 22], *i.e.*, the method that is generally used to deal with the problem. Against this background, we are able to successfully apply to this case the alternative SPDERG approach to boundary layer problems [14, 15], by clarifying similarities and differences.

The procedure that we choose for applying the approach makes use of a basic observation in [15], that the bare quantities to be effectively renormalized are the ICVs of the problem (in fact, here, the substrate one). By starting from the 1st order ODEs to be obeyed by the inner solutions, with ICVs given at a generic time, and by performing the calculations up to the 2nd order, besides generally outlining the contribution of the single terms in the functions to the behaviours captured by the obtained approximations, we are able to show that one gets exactly the same outer component, as in the standard PE 1st order UA, for the substrate (thus, as expected, also for the complex).

First of all, this result was not to be taken for granted. In fact, in the context of the boundary layer problems studied in the original works [15], the approach turned out to be able to possibly

give not exactly the same terms as in the corresponding PE UA, but anyway a better approximation in the matching region. Therefore, we interpret the outcome of the present study also as a further confirmation, from a very different point of view, of the correctness of the known PE results up to 1st order.

At the same time, within our application, the SPDERG approach turns out to make possible to correctly manage both the secular terms and the constant ones, without the imposition of MCs (that in the present case, already at the 1st order, need in fact to be two term conditions, that involve the first derivatives of the outer solutions, too [4]).

Actually, one can see an analogy between the present approach and the MCs of the standard method, since the key ingredient here, *i.e.*, the fact that the renormalized part of the solution has to be independent of the arbitrary time  $\lambda$ , appears similar to the imposition of the MCs at an unknown time. Then, one notices that, when imposing the MCs in the standard PE method, the one for the substrate needs to apply to the complex, too. Thus, within this context, it appears expectable that, as we verified, the ODE to be obeyed by the renormalized substrate ICV is the only one to be obtained. In detail, we verified that the one found from the study of the substrate is the same as the one found in the case of the complex.

On the one hand, within the SPDERG approach, it is necessary to perform the calculations up to the 2nd order for recovering the 1st order outer contribution of the standard PE method. On the other hand, this allowed us to present the 2nd order contribution of the inner solutions for the first time to our knowledge. Moreover, this also allowed us to hypothesize, by assuming that the observed solution behaviours could be iterated, that the constant terms at a given order, not to be renormalized, play the role of a part of the outer component at the following order. Indeed, this appears equivalent to both the imposition of the first term of the MC and the need for cancelling one of the constant terms that appear twice in the UA, within the PE. In fact, it is reasonable to expect that this is a general characteristic of the SPDERG approach to boundary layer problems, at least in the present kind of application procedure, in which one directly renormalizes the bare ICVs. In particular, this observation allowed us to propose refined SPDERG 2nd order UAs, that contain the parts of the 2nd order outer components that are predictable on the basis of our hypothesis, and that are thus asymptotically vanishing, too.

The conclusive comparison among the best different approximations that we considered, in the more demanding case that we studied (the one with the larger value of the expansion parameter,  $\varepsilon = \varepsilon^b = 0.5$ ), shows that the time region in which the SPDERG approach at the 2nd order works better than the PE method at the 1st order, definitely encompasses the matching region. In fact, the obtained SPDERG approximations are nearly indistinguishable, within our numerical precision, from the correct solutions of the problem in about the first 15% of the relevant time window. In detail, the extension of this region is slightly different for the substrate and for the complex, and it is in both of the cases larger when considering the refined UAs. Moreover, in the case of the complex, this last refined approximation works at least as well as the PE 1st order UAs in a part of the relevant time window that also includes the large times.

Actually, we studied particular demanding cases of MM kinetics. In fact, with the present kinetic constant choice, in logarithmic time scale, the curve for the substrate displays three inflection points instead of a single one already for values of the expansion parameter as small as  $\varepsilon = 0.1$ . This observation appears related to an ICV for the sQSSA to the complex behaviour that, apart from being as usual larger than zero, is even larger than the maximum reached by the correct solution for this quantity. Indeed, these qualitative observations would deserve a more careful study, that could allow a better understanding of the parameter's dependence of the relevant time scales in MM kinetics.

From a different point of view, an interesting advantage of the present procedure for applying the SPDERG approach is that, at the 2nd order, one needs to solve a simpler ODE for the 1st order outer component than the corresponding one in the standard method (just because the other part of the outer contribution is already known). This could turn out to be particularly useful when attempting to apply the same procedure within the different framework of the tQSSA since, despite of this framework being more largely applicable from the experimental point of view, the outer solution is not known explicitly at the 1st order and at the 0th order (in any event, it is not known in terms of the Lambert function) [10, 11].

Finally, we notice that, despite of their being quite technical and cumbersome, there is no particular difficulty in the present calculations. The performed detailed verifications, apart from being possibly better formalized in the future, can be in any event taken for granted in other similar cases, too. Thus,

the present results definitely support further applications of the SPDERG approach to boundary layer problems.

## Acknowledgments

The analytical calculations have been partially made with the help of *Mathematica*<sup>®</sup>, whereas the numerical computations have been made by using *Matlab*<sup>®</sup>. We acknowledge interesting discussions with Emanuele Raccach, Gavriel Segre and Pierluigi Vellucci.

## Appendix A

We report the solution  $\tilde{c}_1^{rg}(\tau)$  of the system (31), *i.e.*, the 1st order inner solution for the adimensional complex concentration in MM kinetics within the SPDERG approach, in which the initial values, to be renormalized, are fixed to  $\tilde{s}^{rg}(\tau_0) = \tilde{s}_0^* + \varepsilon \tilde{s}_1^*$  and  $\tilde{c}^{rg}(\tau_0) = \tilde{c}_0^* + \varepsilon \tilde{c}_1^*$ , at  $\tau = \tau_0$ . In detail, we distinguish the terms corresponding to different functions of  $(\tau - \tau_0)$ . We find:

$$\begin{aligned}\tilde{c}_1^{rg}(\tau) &= A_{c_1^{rg}}(\tilde{s}_0^*)(\tau - \tau_0) + B_{c_1^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*, \tilde{c}_0^*) + C_{c_1^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*, \tilde{c}_0^*, \tilde{c}_1^*)e^{-(\tilde{s}_0^* + M)(\tau - \tau_0)} + \\ &+ \left[ D_{c_1^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*, \tilde{c}_0^*)(\tau - \tau_0) + E_{c_1^{rg}}(\tilde{s}_0^*, \tilde{c}_0^*)(\tau - \tau_0)^2 \right] e^{-(\tilde{s}_0^* + M)(\tau - \tau_0)} + \\ &+ F_{c_1^{rg}}(\tilde{s}_0^*, \tilde{c}_0^*)e^{-2(\tilde{s}_0^* + M)(\tau - \tau_0)},\end{aligned}\quad (62)$$

with:

$$\begin{aligned}A_{c_1^{rg}}(\tilde{s}_0^*) &= -\frac{M(M-m)}{(\tilde{s}_0^* + M)^3}\tilde{s}_0^*; \\ B_{c_1^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*, \tilde{c}_0^*) &= -\frac{M(\tilde{s}_0^* - M + 2m)}{(\tilde{s}_0^* + M)^4}\tilde{s}_0^* + \frac{M}{(\tilde{s}_0^* + M)^2}\tilde{s}_1^* + \frac{M(\tilde{s}_0^* + m)}{(\tilde{s}_0^* + M)^3}\tilde{c}_0^*; \\ C_{c_1^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*, \tilde{c}_0^*, \tilde{c}_1^*) &= \tilde{c}_1^* + \frac{M(\tilde{s}_0^* - M + 2m)}{(\tilde{s}_0^* + M)^4}\tilde{s}_0^* + \frac{\tilde{s}_0^* + m}{(\tilde{s}_0^* + M)^4}(\tilde{s}_0^*)^2 - \frac{M}{(\tilde{s}_0^* + M)^2}\tilde{s}_1^* + \\ &- \frac{(2\tilde{s}_0^* + M)(\tilde{s}_0^* + m)}{(\tilde{s}_0^* + M)^3}\tilde{c}_0^* + \frac{\tilde{s}_0^* + m}{(\tilde{s}_0^* + M)^2}(\tilde{c}_0^*)^2; \\ D_{c_1^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*, \tilde{c}_0^*) &= -\frac{(\tilde{s}_0^* + m)(\tilde{s}_0^* - M)}{(\tilde{s}_0^* + M)^3}\tilde{s}_0^* + \frac{\tilde{s}_0^*\tilde{s}_1^*}{\tilde{s}_0^* + M} - \frac{\tilde{s}_1^*\tilde{c}_0^*}{(\tilde{s}_0^* + M)^2} + \frac{(\tilde{s}_0^* + m)(2\tilde{s}_0^* - M)}{(\tilde{s}_0^* + M)^2}\tilde{c}_0^* + \\ &- \frac{\tilde{s}_0^* + m}{(\tilde{s}_0^* + M)^2}(\tilde{c}_0^*)^2; \\ E_{c_1^{rg}}(\tilde{s}_0^*, \tilde{c}_0^*) &= -\frac{(M-m)}{2(\tilde{s}_0^* + M)^2}(\tilde{s}_0^*)^2 + \frac{(M-m)}{2(\tilde{s}_0^* + M)}\tilde{s}_0^*\tilde{c}_0^*; \\ F_{c_1^{rg}}(\tilde{s}_0^*, \tilde{c}_0^*) &= -\frac{\tilde{s}_0^* + m}{(\tilde{s}_0^* + M)^4}(\tilde{s}_0^*)^2 + 2\frac{\tilde{s}_0^* + m}{(\tilde{s}_0^* + M)^3}\tilde{s}_0^*\tilde{c}_0^* - \frac{\tilde{s}_0^* + m}{(\tilde{s}_0^* + M)^2}(\tilde{c}_0^*)^2.\end{aligned}\quad (63)$$

In particular, one can check that one has  $\tilde{c}_1^{rg}(\tau_0) = B_{c_1^{rg}} + C_{c_1^{rg}} + F_{c_1^{rg}} = \tilde{c}_1^*$ . Moreover, for  $\tilde{s}_0^* = 1$ ,  $\tilde{s}_1^* = \tilde{c}_0^* = \tilde{c}_1^* = 0$  and  $\tau_0 = 0$ , the result on  $\tilde{c}_1^{in}$  reported in (18) is correctly reproduced.

## Appendix B

We report the solutions  $\tilde{s}_2^{rg}(\tau)$  and  $\tilde{c}_2^{rg}(\tau)$  of the system (46), *i.e.*, the 2nd order inner solution for the adimensional substrate and complex concentrations in MM kinetics within the SPDERG approach, in which the ICVs are anyway already fixed, for the sake of simplicity, to  $\tilde{s}^{rg}(\tau_0) = \tilde{s}_0^* + \varepsilon \tilde{s}_1^*$  and  $\tilde{c}^{rg}(\tau_0) = 0$ , at  $\tau = \tau_0$ . As discussed in the text, this choice is done on the basis of the expectation, to be verified, that the only quantities that need to be renormalized at the present order are  $\tilde{s}_0^*$  (in agreement with the previously obtained result) and  $\tilde{s}_1^*$ .

Let us start with  $\tilde{s}_2^{rg}(\tau)$ . Notice that here we separate both the terms that are different functions of  $(\tau - \tau_0)$  and the terms that depend only on  $\tilde{s}_0^*$  or both on  $\tilde{s}_0^*$  and on  $\tilde{s}_1^*$ :

$$\begin{aligned}\tilde{s}_2^{rg}(\tau) &= \left[ A_{s_2^{rg}}(\tilde{s}_0^*) + B_{s_2^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*) \right] (\tau - \tau_0) + C_{s_2^{rg}}(\tilde{s}_0^*)(\tau - \tau_0)^2 + D_{s_2^{rg}}(\tilde{s}_0^*) + E_{s_2^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*) + \\ &+ \left\{ \left[ F_{s_2^{rg}}(\tilde{s}_0^*) + G_{s_2^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*) \right] + \left[ H_{s_2^{rg}}(\tilde{s}_0^*) + I_{s_2^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*) \right] (\tau - \tau_0) \right\} e^{-(\tilde{s}_0^* + M)(\tau - \tau_0)} + \\ &+ J_{s_2^{rg}}(\tilde{s}_0^*)(\tau - \tau_0)^2 e^{-(\tilde{s}_0^* + M)(\tau - \tau_0)} + K_{s_2^{rg}}(\tilde{s}_0^*)e^{-2(\tilde{s}_0^* + M)(\tau - \tau_0)}.\end{aligned}\quad (64)$$

In fact, only the first three terms contribute to the part to be renormalized of the whole functions,  $\tilde{s}_2^{rg}(\tau)$ , at the 2nd order. Their coefficients are:

$$A_{s_2^{rg}}(\tilde{s}_0^*) = \frac{2M(M-m)\tilde{s}_0^*}{(\tilde{s}_0^* + M)^4}(\tilde{s}_0^* + m); \quad B_{s_2^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*) = -\frac{M(M-m)\tilde{s}_1^*}{(\tilde{s}_0^* + M)^2};$$

$$C_{s_2^{rg}}(\tilde{s}_0^*) = \frac{M(M-m)^2\tilde{s}_0^*}{2(\tilde{s}_0^*+M)^3}. \quad (65)$$

For the sake of completeness, we give the explicit dependence on  $\tilde{s}_0^*$  and  $\tilde{s}_1^*$  also of the other terms. One finds:

$$\begin{aligned} D_{s_2^{rg}}(\tilde{s}_0^*) &= \frac{\tilde{s}_0^*}{2(\tilde{s}_0^*+M)^5} [(\tilde{s}_0^*)^2(3M-m) - \tilde{s}_0^*(2M^2-5Mm-m^2) - 2Mm(2M-3m)]; \\ E_{s_2^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*) &= -G_{s_2^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*) = -\frac{\tilde{s}_1^*}{(\tilde{s}_0^*+M)^3} [\tilde{s}_0^*(2M-m) + Mm]; \\ F_{s_2^{rg}}(\tilde{s}_0^*) &= -\frac{\tilde{s}_0^*}{(\tilde{s}_0^*+M)^5} [(\tilde{s}_0^*)^3 + (\tilde{s}_0^*)^2(2M+m) - \tilde{s}_0^*(M^2-3Mm-m^2) - Mm(2M-3m)]; \\ H_{s_2^{rg}}(\tilde{s}_0^*) &= \frac{\tilde{s}_0^*}{(\tilde{s}_0^*+M)^4} [(\tilde{s}_0^*)^3 - (\tilde{s}_0^*)^2(M-2m) - \tilde{s}_0^*M^2 - Mm^2]; \\ I_{s_2^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*) &= -\frac{\tilde{s}_0^*\tilde{s}_1^*}{(\tilde{s}_0^*+M)^2}(\tilde{s}_0^*+m); \quad J_{s_2^{rg}}(\tilde{s}_0^*) = \frac{(\tilde{s}_0^*)^2(M-m)}{2(\tilde{s}_0^*+M)^3}(\tilde{s}_0^*+m); \\ K_{s_2^{rg}}(\tilde{s}_0^*) &= \frac{(\tilde{s}_0^*)^2(\tilde{s}_0^*+m)}{2(\tilde{s}_0^*+M)^5}(2\tilde{s}_0^*+M+m). \end{aligned} \quad (66)$$

One can check that  $\tilde{s}_2^{rg}(\tau_0) = D_{s_2^{rg}} + E_{s_2^{rg}} + F_{s_2^{rg}} + G_{s_2^{rg}} + K_{s_2^{rg}} = 0$ . Notice moreover that, when calculating the  $\mathcal{R}_2^s(\tau)$  contribution to the SPDERG 2nd order UA to the correct solution, with the renormalized divergent part, one is interested in evaluating this quantity in  $\tau_0 = 0$ , for  $\tilde{s}_0^* = 1$  and  $\tilde{s}_1^* = 0$ . Hence, one immediately gets  $E_{s_2^{rg}} = G_{s_2^{rg}} = I_{s_2^{rg}} = 0$ , since all of these coefficients are proportional to  $\tilde{s}_1^*$ . The other coefficients can be easily calculated for  $\tilde{s}_0^* = 1$ , and the corresponding  $\mathcal{R}_2^s$  is reported in (56).

In the case of  $\tilde{c}_2^{rg}(\tau)$ , since the complete formula is definitely uselessly cumbersome, we limit ourselves to report explicitly the dependence on  $\tilde{s}_0^*$  and  $\tilde{s}_1^*$  only in the part of the whole function that belongs to the one to be renormalized (*i.e.*, in the coefficients of the terms that are proportional to  $(\tau - \tau_0)$  and to  $(\tau - \tau_0)^2$ ).

Correspondingly, we write from the beginning:

$$\tilde{c}_2^{rg}(\tau) = \tilde{c}_{2,div}^{rg}(\tau) + \mathcal{R}_2^c(\tau). \quad (67)$$

Here, we are collecting in  $\tilde{c}_{2,div}^{rg}(\tau)$  the terms that contribute to the part of the 2nd order complex function to be renormalized:

$$\tilde{c}_{2,div}^{rg}(\tau) = [A_{c_{2,div}^{rg}}(\tilde{s}_0^*) + B_{c_{2,div}^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*)](\tau - \tau_0) + C_{c_{2,div}^{rg}}(\tilde{s}_0^*)(\tau - \tau_0)^2, \quad (68)$$

with, in detail:

$$\begin{aligned} A_{c_{2,div}^{rg}}(\tilde{s}_0^*) &= -\frac{M(M-m)\tilde{s}_0^*}{(\tilde{s}_0^*+M)^6} [2(\tilde{s}_0^*)^2 - 5\tilde{s}_0^*(M-m) + M^2 - 3Mm]; \\ B_{c_{2,div}^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*) &= \frac{M(M-m)\tilde{s}_1^*}{(\tilde{s}_0^*+M)^4}(2\tilde{s}_0^*-M); \quad C_{c_{2,div}^{rg}}(\tilde{s}_0^*) = -\frac{M(M-m)^2\tilde{s}_0^*}{2(\tilde{s}_0^*+M)^5}(2\tilde{s}_0^*-M). \end{aligned} \quad (69)$$

On the other hand,  $\mathcal{R}_2^c(\tau)$  contains all the terms that remain constant or tend to zero in the large  $(\tau - \tau_0)$  limit, and it gives the 2nd order contribution of the inner solution to the SPDERG UA to the correct complex function (that is  $O(\varepsilon^2)$ ). For  $\tilde{s}_0^* = 1$  and  $\tilde{s}_1^* = 0$ , by also coherently evaluating it in  $\tau_0 = 0$ , one finds:

$$\begin{aligned} \mathcal{R}_2^c(\tau) \Big|_{\substack{\tilde{s}_0^*=1 \\ \tilde{s}_1^*=0}} &= A_{\mathcal{R}_2^c} + [B_{\mathcal{R}_2^c} + C_{\mathcal{R}_2^c}\tau + D_{\mathcal{R}_2^c}\tau^2 + E_{\mathcal{R}_2^c}\tau^3 + F_{\mathcal{R}_2^c}\tau^4] e^{-(1+M)\tau} + \\ &+ [G_{\mathcal{R}_2^c} + H_{\mathcal{R}_2^c}\tau + I_{\mathcal{R}_2^c}\tau^2] e^{-2(1+M)\tau} + J_{\mathcal{R}_2^c} e^{-3(1+M)\tau}. \end{aligned} \quad (70)$$

In fact, it is useful to report also the complete dependence on  $\tilde{s}_0^*$ ,  $\tilde{s}_1^*$ ,  $M$  and  $m$  of the coefficient  $A_{\mathcal{R}_2^c}$  (the constant term in the original  $\tilde{c}_2^{rg}(\tau)$ ), since it allows to propose a refined SPDERG 2nd order UA to the correct solution. We find:

$$\begin{aligned} A_{\mathcal{R}_2^c}(\tilde{s}_0^*, \tilde{s}_1^*) &= -\frac{M(\tilde{s}_0^*)^4}{(\tilde{s}_0^*+M)^7} + \frac{M(\tilde{s}_0^*)^3(9M-11m)}{2(\tilde{s}_0^*+M)^7} - \frac{M(\tilde{s}_0^*)^2}{2(\tilde{s}_0^*+M)^7}(12M^2-27Mm+13m^2) + \\ &+ \frac{M^2\tilde{s}_0^*(M^2-6mM+6m^2)}{(\tilde{s}_0^*+M)^7} + \frac{M\tilde{s}_1^*}{(\tilde{s}_0^*+M)^5}[2(\tilde{s}_0^*)^2 - \tilde{s}_0^*(5M-6m) + M(1-2m)] + \\ &- \frac{M(\tilde{s}_1^*)^2}{(\tilde{s}_0^*+M)^3}, \end{aligned} \quad (71)$$

whereas the dependence on  $M$  and  $m$  of all the coefficients, calculated in  $\tilde{s}_0^* = 1$  and  $\tilde{s}_1^* = 0$ , is given by:

$$\begin{aligned} A_{\mathcal{R}_2^c} &= \frac{M^4-6M^3(m+1)}{(1+M)^7} + \frac{3M^2(4m^2+9m+3)}{2(1+M)^7} - \frac{M(13m^2+11m+2)}{2(1+M)^7}; \\ B_{\mathcal{R}_2^c} &= -\frac{M^4-6M^3(m+1)}{(1+M)^7} - \frac{M^2(6m^2+10m+3)}{(1+M)^7} + \frac{M(m-7)-9m^2-m}{4(1+M)^7}; \\ C_{\mathcal{R}_2^c} &= \frac{M^3(2m+1)}{(1+M)^6} - \frac{M^2(3m^2+7m+5)}{(1+M)^6} + \frac{M(12m^2+15m+5)}{2(1+M)^6} + \frac{3m^2+3m+2}{2(1+M)^6}; \\ D_{\mathcal{R}_2^c} &= -\frac{1}{2(1+M)^5}[2M^3+M^2(m^2-6m-3)+M(4m^2-m-3)+2m^2+3m+1]; \end{aligned}$$

$$\begin{aligned}
E_{\mathcal{R}_2^c} &= \frac{(M-m)}{6(1+M)^4} [M^2 + M(2m+3) - 3(m+1)]; & F_{\mathcal{R}_2^c} &= -\frac{(M-m)^2}{8(1+M)^3}; \\
G_{\mathcal{R}_2^c} &= -\frac{M^2(7m+3)}{2(1+M)^7} + \frac{M(13m^2+11m+6)}{2(1+M)^7} + \frac{3m^2+2m+1}{(1+M)^7}; \\
H_{\mathcal{R}_2^c} &= \frac{1}{(1+M)^6} [M^2 + M(2m^2+m+1) - 3m-2]; \\
I_{\mathcal{R}_2^c} &= -\frac{(M-m)}{(1+M)^5} (m+1); & J_{\mathcal{R}_2^c} &= -\frac{(m+1)}{4(1+M)^7} (M+3m+4).
\end{aligned} \tag{72}$$

One can check that  $\mathcal{R}_2^c(0) = A_{\mathcal{R}_2^c} + B_{\mathcal{R}_2^c} + G_{\mathcal{R}_2^c} + J_{\mathcal{R}_2^c} = 0$ , correctly.

## Appendix C

Let us relabel  $T_1(\tilde{s}_0^*)$ ,  $T_2(\tilde{s}_0^*)$ ,  $T_3(\tilde{s}_0^*, \tilde{s}_1^*)$  and  $T_4(\tilde{s}_0^*)$  the coefficients of the first four terms in  $\tilde{c}_{div}^{rg}(\tau)$  at the 2nd order given in (58). Hence, one has:

$$\begin{aligned}
\tilde{c}_{div}^{rg}(\tau) &= T_1(\tilde{s}_0^*) + \varepsilon T_2(\tilde{s}_0^*) + \varepsilon T_3(\tilde{s}_0^*, \tilde{s}_1^*) + \varepsilon T_4(\tilde{s}_0^*)(\tau - \tau_0) + \\
&+ \varepsilon^2 A_{c_{2,div}^{rg}}(\tilde{s}_0^*)(\tau - \tau_0) + \varepsilon^2 B_{c_{2,div}^{rg}}(\tilde{s}_0^*, \tilde{s}_1^*)(\tau - \tau_0) + \varepsilon^2 C_{c_{2,div}^{rg}}(\tilde{s}_0^*)(\tau - \tau_0)^2,
\end{aligned} \tag{73}$$

with:

$$\begin{aligned}
T_1(\tilde{s}_0^*) &= \frac{\tilde{s}_0^*}{\tilde{s}_0^* + M}; & T_2(\tilde{s}_0^*) &= -\frac{M(\tilde{s}_0^* - M + 2m)}{(\tilde{s}_0^* + M)^4} \tilde{s}_0^*; \\
T_3(\tilde{s}_0^*, \tilde{s}_1^*) &= \frac{M\tilde{s}_1^*}{(\tilde{s}_0^* + M)^2}; & T_4(\tilde{s}_0^*) &= -\frac{M(M-m)\tilde{s}_0^*}{(\tilde{s}_0^* + M)^3};
\end{aligned} \tag{74}$$

Here, in detail:  $T_1$  is the coefficient of the 0th order term that was already present in  $\tilde{c}_0^{rg}(\tau)$  given in (30);  $T_2 + T_3 = B_{c_1^{rg}}$  with  $B_{c_1^{rg}}$  (calculated in  $\tilde{c}_0^* = 0$ ) the constant term in  $\tilde{c}_1^{rg}(\tau)$  given in (63);  $T_4 = A_{c_1^{rg}}$ , with  $A_{c_1^{rg}}$  the coefficient of the single 1st order secular term in  $\tilde{c}_1^{rg}(\tau)$  given again in (63);  $A_{c_{2,div}^{rg}}$ ,  $B_{c_{2,div}^{rg}}$  and  $C_{c_{2,div}^{rg}}$  are the coefficients of the three 2nd order secular terms in  $\tilde{c}_2^{rg}(\tau)$  given in (69), respectively.

When renormalizing the bare constants by  $\tilde{s}_0^* = (1 + \varepsilon z_{s_0,1} + \varepsilon^2 z_{s_0,2})\tilde{s}_0^{rg*}$  and  $\tilde{s}_1^* = (1 + \varepsilon z_{s_0,1})\tilde{s}_1^{rg*}$ , respectively (with the already chosen  $z_{s_0,1}$  given in (35) and  $z_{s_0,2}$ ,  $z_{s_1,1}$  given in (51)), one finds (up to order  $\varepsilon^2$ ):

$$\begin{aligned}
T_1(\tilde{s}_0^*) - T_1(\tilde{s}_0^{rg*}) &= \left[ \frac{dT_1(\tilde{s}_0^*)}{d\tilde{s}_0^*} \Big|_{\tilde{s}_0^* = \tilde{s}_0^{rg*}} \right] (\varepsilon z_{s_0,1} + \varepsilon^2 z_{s_0,2}) \tilde{s}_0^{rg*} + \frac{1}{2} \left[ \frac{d^2 T_1(\tilde{s}_0^*)}{d(\tilde{s}_0^*)^2} \Big|_{\tilde{s}_0^* = \tilde{s}_0^{rg*}} \right] (\varepsilon z_{s_0,1})^2 (\tilde{s}_0^{rg*})^2 = \\
&= \varepsilon \frac{M(M-m)\tilde{s}_0^{rg*}}{(\tilde{s}_0^{rg*} + M)^3} (\lambda - \tau_0) - \varepsilon^2 Mm \frac{(M-m)\tilde{s}_0^{rg*}}{(\tilde{s}_0^{rg*} + M)^5} (\lambda - \tau_0) + \\
&- \varepsilon^2 M \frac{(M-m)^2 \tilde{s}_0^{rg*}}{2(\tilde{s}_0^{rg*} + M)^5} (2\tilde{s}_0^{rg*} - M)(\lambda - \tau_0)^2 = \\
&= -\varepsilon T_4(\tilde{s}_0^{rg*})(\lambda - \tau_0) - \varepsilon^2 Mm \frac{(M-m)\tilde{s}_0^{rg*}}{(\tilde{s}_0^{rg*} + M)^5} (\lambda - \tau_0) + \varepsilon^2 C_{c_{2,div}^{rg}}(\tilde{s}_0^{rg*})(\lambda - \tau_0)^2;
\end{aligned} \tag{75}$$

$$\begin{aligned}
\varepsilon [T_2(\tilde{s}_0^*) - T_2(\tilde{s}_0^{rg*})] &= \varepsilon \left[ \frac{dT_2(\tilde{s}_0^*)}{d\tilde{s}_0^*} \Big|_{\tilde{s}_0^* = \tilde{s}_0^{rg*}} \right] (\varepsilon z_{s_0,1}) \tilde{s}_0^{rg*} = \\
&= \varepsilon^2 \frac{M(M-m)\tilde{s}_0^{rg*}}{(\tilde{s}_0^{rg*} + M)^6} [2(\tilde{s}_0^{rg*})^2 - (5M-6m)\tilde{s}_0^{rg*} + M(M-2m)] (\lambda - \tau_0) = \\
&= -\varepsilon^2 A_{c_{2,div}^{rg}}(\tilde{s}_0^{rg*})(\lambda - \tau_0) + \varepsilon^2 Mm \frac{(M-m)\tilde{s}_0^{rg*}}{(\tilde{s}_0^{rg*} + M)^5} (\lambda - \tau_0);
\end{aligned} \tag{76}$$

$$\begin{aligned}
\varepsilon [T_3(\tilde{s}_0^*, \tilde{s}_1^*) - T_3(\tilde{s}_0^{rg*}, \tilde{s}_1^{rg*})] &= \varepsilon \left[ \frac{\partial T_3(\tilde{s}_0^*, \tilde{s}_1^*)}{\partial \tilde{s}_0^*} \Big|_{\substack{\tilde{s}_0^* = \tilde{s}_0^{rg*} \\ \tilde{s}_1^* = \tilde{s}_1^{rg*}}} \right] (\varepsilon z_{s_0,1}) \tilde{s}_0^{rg*} + \varepsilon \left[ \frac{\partial T_3(\tilde{s}_0^*, \tilde{s}_1^*)}{\partial \tilde{s}_1^*} \Big|_{\substack{\tilde{s}_0^* = \tilde{s}_0^{rg*} \\ \tilde{s}_1^* = \tilde{s}_1^{rg*}}} \right] (\varepsilon z_{s_1,1}) \tilde{s}_1^{rg*} = \\
&= \varepsilon^2 \left[ -\frac{2M(M-m)\tilde{s}_1^{rg*}}{(\tilde{s}_0^{rg*} + M)^4} \tilde{s}_0^{rg*} + \frac{M^2(M-m)}{(\tilde{s}_0^{rg*} + M)^4} \tilde{s}_1^{rg*} \right] (\lambda - \tau_0) = \\
&= -\varepsilon^2 B_{c_{2,div}^{rg}}(\tilde{s}_0^{rg*}, \tilde{s}_1^{rg*})(\lambda - \tau_0);
\end{aligned} \tag{77}$$

$$\begin{aligned}
\varepsilon [T_4(\tilde{s}_0^*) - T_4(\tilde{s}_0^{rg*})] &= \varepsilon \left[ \frac{dT_4(\tilde{s}_0^*)}{d\tilde{s}_0^*} \Big|_{\tilde{s}_0^* = \tilde{s}_0^{rg*}} \right] (\varepsilon z_{s_0,1}) \tilde{s}_0^{rg*} = \varepsilon^2 \frac{M(M-m)^2 \tilde{s}_0^{rg*}}{(\tilde{s}_0^{rg*} + M)^4} (2\tilde{s}_0^{rg*} - M)(\lambda - \tau_0) = \\
&= -2\varepsilon^2 C_{c_{2,div}^{rg}}(\tilde{s}_0^{rg*})(\lambda - \tau_0).
\end{aligned} \tag{78}$$

Therefore, when moreover writing  $(\tau - \tau_0) = (\tau - \lambda) + (\lambda - \tau_0)$ , since obviously  $(\tau - \tau_0)^2 = (\tau - \lambda)^2 + 2(\tau - \lambda)(\lambda - \tau_0) + (\lambda - \tau_0)^2$ , one gets exactly the same form of  $\tilde{c}_{div}^{rg}(\tau, \lambda)$  given in (58), with  $\tau_0 \rightarrow \lambda$ ,  $\tilde{s}_0^* \rightarrow \tilde{s}_0^{rg*}(\lambda)$  and  $\tilde{s}_1^* \rightarrow \tilde{s}_1^{rg*}(\lambda)$ .



Let us now also remind that  $d\tilde{s}_0^{rg*}/d\lambda = -\varepsilon z_{s_0,1}\tilde{s}_0^{rg*}/(\lambda - \tau_0)$ . Correspondingly, one can make partially use again of the previous formulas in the study of the derivative with respect to  $\lambda$  of  $\tilde{c}_{div}^{rg}(\tau, \lambda)$ :

$$\begin{aligned} \frac{d\tilde{c}_{div}^{rg}(\tau, \lambda)}{d\lambda} &= \frac{dT_1(\tilde{s}_0^{rg*})}{d\tilde{s}_0^{rg*}} \frac{d\tilde{s}_0^{rg*}}{d\lambda} + \varepsilon \frac{dT_2(\tilde{s}_0^{rg*})}{d\tilde{s}_0^{rg*}} \frac{d\tilde{s}_0^{rg*}}{d\lambda} + \varepsilon \frac{\partial T_3(\tilde{s}_0^{rg*}, \tilde{s}_1^{rg*})}{\partial \tilde{s}_0^{rg*}} \frac{d\tilde{s}_0^{rg*}}{d\lambda} + \\ &+ \varepsilon \frac{\partial T_3(\tilde{s}_0^{rg*}, \tilde{s}_1^{rg*})}{\partial \tilde{s}_1^{rg*}} \frac{d\tilde{s}_1^{rg*}}{d\lambda} + \varepsilon \frac{dT_4(\tilde{s}_0^{rg*})}{d\tilde{s}_0^{rg*}} \frac{d\tilde{s}_0^{rg*}}{d\lambda} (\tau - \lambda) - \varepsilon T_4(\tilde{s}_0^{rg*}) + \\ &- \varepsilon^2 A_{c_{2,div}^{rg}}(\tilde{s}_0^{rg*}) - \varepsilon^2 B_{c_{2,div}^{rg}}(\tilde{s}_0^{rg*}, \tilde{s}_1^{rg*}) - 2\varepsilon^2 C_{c_{2,div}^{rg}}(\tilde{s}_0^{rg*})(\tau - \lambda). \end{aligned} \quad (79)$$

In detail, one can notice first of all that, to satisfy  $d\tilde{c}_{div}^{rg}(\tau, \lambda)/d\lambda = 0$ , the 1st order known result on  $d\tilde{s}_0^{rg*}/d\lambda$  given in Eqs. (38) needs to be once again satisfied. From this point of view, see in particular Eqs. (75). Indeed, the contribution that comes from the term  $dT_1(\tilde{s}_0^{rg*})/d\tilde{s}_0^{rg*}$  in the same way as the first term in that formula and the one that is equal to  $-\varepsilon T_4(\tilde{s}_0^{rg*})$  are the only ones to be proportional to  $\varepsilon$ . Thus, they obviously need to cancel each other. Then, the term involving the derivative of  $T_2$  is partially cancelled by the one proportional to  $A_{c_{2,div}^{rg}}$ , leaving a contribution equal to  $-\varepsilon^2 Mm(M-m)\tilde{s}_0^{rg*}/(\tilde{s}_0^{rg*} + M)^5$ , as can be seen from Eqs. (76). Moreover, the two terms proportional to  $(\tau - \lambda)$  cancel each other, too, as can be seen from Eqs. (78).

Therefore, by using  $d\tilde{s}_0^{rg*}(\lambda)/d\lambda = -\varepsilon(M-m)\tilde{s}_0^{rg*}/(\tilde{s}_0^{rg*} + M)$ , by imposing the scaling condition  $d\tilde{c}_{div}^{rg}(\tau, \lambda)/d\lambda = 0$  at the 2nd order, and by making partially use of Eqs. (77), we end up with the equation:

$$-\varepsilon \frac{Mm(M-m)\tilde{s}_0^{rg*}}{(\tilde{s}_0^{rg*} + M)^5} + \varepsilon \frac{2M(M-m)\tilde{s}_0^{rg*}\tilde{s}_1^{rg*}}{(\tilde{s}_0^{rg*} + M)^4} + \frac{M}{(\tilde{s}_0^{rg*} + M)^2} \frac{d\tilde{s}_1^{rg*}}{d\lambda} - \varepsilon \frac{M(M-m)\tilde{s}_1^{rg*}}{(\tilde{s}_0^{rg*} + M)^4} (2\tilde{s}_0^{rg*} - M) = 0. \quad (80)$$

Hence, we get the expected result:

$$\frac{d\tilde{s}_1^{rg*}}{d\lambda} = \varepsilon \left[ \frac{m(M-m)\tilde{s}_0^{rg*}}{(\tilde{s}_0^{rg*} + M)^3} - \frac{M(M-m)\tilde{s}_1^{rg*}}{(\tilde{s}_0^{rg*} + M)^2} \right], \quad (81)$$

that completes the present verification. Indeed, this is the same ODE to be obeyed by  $\tilde{s}_1^{rg*}(\lambda)$  that we previously obtained in the study of the substrate, and that is reported in Eqs. (53).

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